Glycaemic index and glycaemic response: exploring an optimum profile for cognitive function across the day

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Foods associated with a more favourable glycaemic response have been linked with cognitive benefits in acute settings in both the clinically healthy and those with type 2 diabetes. However, the current evidence is largely limited to examination of the effects of a single meal, particularly breakfast. Given that the daily human dietary pattern consists of multiple meals, the single meal paradigm fails to provide information about the glycaemic response and cognitive performance throughout the day. In addition, the glycaemic response following a meal has been shown to influence postprandial glycaemia and cognitive performance following a subsequent meal, which further illustrates the need to extend the current single meal investigation to a multiple meal testing paradigm. This thesis, therefore, aimed to investigate the relationship between the glycaemic response, cognitive performance and subjective mood across three consecutive meals in both clinically healthy and T2DM populations.

Initially, two novel test meal profiles were designed using the glycaemic index concept with the aim of producing significantly different glycaemic profiles across the day; specifically, a Favourable (FGP) and Unfavourable (UGP) Glycaemic Profile. The two glycaemic conditions were then implemented in two randomised cross-over intervention studies in clinically healthy (n=40) and T2DM populations (n=25) to examine their effects on cognitive performance and subjective mood. Finally, a post-hoc analysis was performed to compare the glycaemic and cognitive outcome responses of the clinically healthy and T2DM populations. The FGP meals consistently produced a lower glycaemic response across the day in all samples. The clinically healthy population did not gain cognitive benefits from either condition, whereas the T2DM population displayed sustained cognitive performance during the FGP condition, particularly during the period after breakfast consumption. In addition, poor glucose regulators (defined via a glucose composite score) displayed worse cognitive performance than good glucose regulators within each population under an increased cognitive load. Finally, the glycaemic conditions had minimal impact on subjective mood. Taken together, these findings indicate that a more favourable glycaemic response profile over the course of several meals is associated with better cognitive performance in those with poorer glucose tolerance (i.e. T2DM) compared to an unfavourable glycaemic response profile. Longitudinal investigation of glycaemic control and cognitive performance in those with T2DM, using the multiple meal profiles developed in this thesis is suggested as future research. This would provide data on the extent, if any, of whether a long-term favourable glycaemic profile is associated with attenuation of cognitive impairment observed in those with T2DM.

Table of Contents

Table of Figures

Table of Tables

Abbreviations

Ach	Acetylcholine
ADA	American Diabetes Association
AGEs	Advanced Glycation End Products
AUC	Area Under the Curve
BBB	Blood Brain Barrier
BMI	Body Mass Index
BP	Blood Pressure
CGM	Continuous Glucose Monitoring
CHO	Carbohydrate
CRT	Choice Reaction Time
CVD	Cardiovascular Disease
FFAs	Free Fatty Acids
FG	Fasting Glucose
FGP	Favourable Glycaemic Profile
GI	Glycaemic Index
GL	Glycaemic Load
GTT	Glucose Tolerance Test
Hb1Ac	Glycated Haemoglobin
HGI	High Glycaemic Index
HGL	High Glycaemic Load
HPA	Hypothalamic Pituitary-Adrenal axis
iAUC	Incremental Area Under the Curve
IEC	International Expert Committee
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
ISF	Interstitial Fluid

ISI Inter-Stimulus Interval

LGI Low Glycaemic Index

LGL Low Glycaemic Load

LM Letter Memory

NGT Normal Glucose Tolerance

OGTT Oral Glucose Tolerance Test

PMd Dorsal Pre-Motor area

PMv Ventral Pre-Motor area

RGT Regulator Type

RT Reaction Time

RVIP Rapid Visual Information Processing

T2DM Type 2 Diabetes Mellitus

tAUC Total Area Under the Curve

VAS Visual Analogue Scale

UGP Unfavourable Glycaemic Profile

WHO World Health Organisation

Chapter 1

General Introduction

1.1 Glucose and cognitive performance

Glucose is the key energy source for humans, providing approximately 3.75 kilocalories of energy per gram consumed (Maclean et al., 2003). The brain, which consumes a high amount of energy relative to the rest of the body, uses glucose as the primary source for metabolic energy (Amiel, 1994; Gomez-Pinilla, 2008; Seiber & Traystman, 1992). Due to the brain possessing minimal glycogen stores, neural energy requirements must be met almost solely with exogenous glucose via the blood (Weiss, 1986). In humans, exogenous glucose is generally obtained by consuming foods containing carbohydrates (CHO). This has been the motivation for a wealth of research investigating the link between glucose consumption and cognitive performance. The majority of early studies have compared cognitive performance following glucose ingestion (via food consumption) relative to glucose omission. The consumption of glucose has been reported to show benefits in various cognitive domains such as reaction time, attention and verbal memory (Benton & Parker, 1998; Hoyland et al., 2008; Messier et al., 2004). Such benefits have been found in a range of populations from healthy individuals (King et al, 1945; Kaplan et al, 2000; Defeyter & Russo, 2013) to those with poor glucose regulation (Korol and Gold, 1998; Smith et al, 1994; Messier et al, 1999).

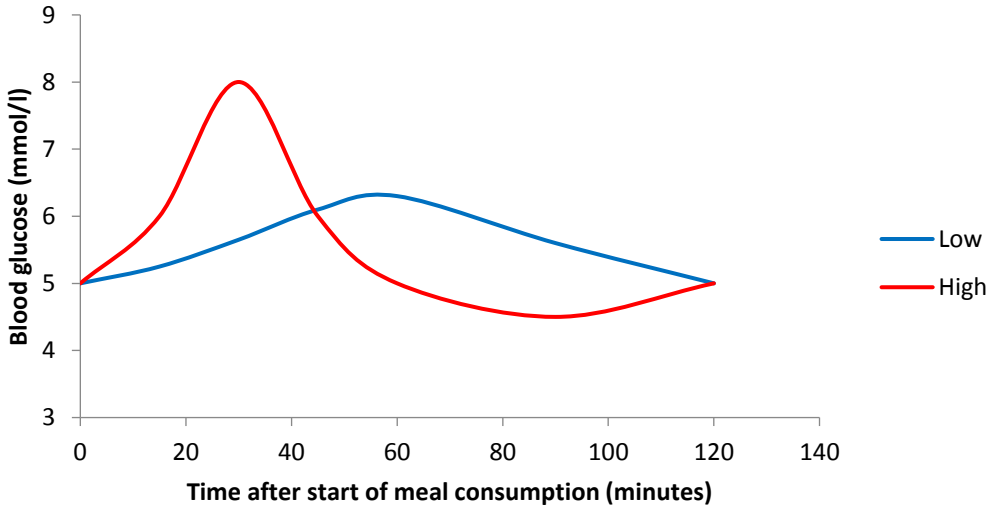
Although there is a general consensus that glucose consumption is beneficial for cognitive performance compared to glucose omission, research suggests that blood glucose levels and cognitive performance do not share a perfect positive relationship (Messier, 2004). Therefore, better cognitive performance cannot be predicted by continually increasing blood glucose levels, with evidence suggesting that hyperglycaemia (high blood glucose levels) is associated with cognitive impairments in areas such as attention and working memory (Sommerfield, Deary & Frier, 2004). Recent literature has suggested that an association between the glycaemic response to CHO consumption and cognitive performance exists. The “glycaemic response” is defined as the effect that a food or meal has on blood glucose levels after consumption. Considerable research has examined the relationship between postprandial glycaemic response and cognitive performance (see Chapter 2 for a review).

1.2 Glycaemic response, glucose tolerance and cognitive performance

When an individual consumes a CHO-containing food, a glycaemic response will follow. This response can be a low glycaemic response, characterised by a slow increase in blood glucose levels to a low and sustained peak followed by a steady decline back to fasting levels (see Figure 1.1). Conversely, the response can be a high glycaemic response, characterised by a sharp increase in blood glucose levels to a high peak followed by a rapid decline which can result in reactive hypoglycaemia before returning back to fasting levels (see Figure 1.1). The shape of this glycaemic response is determined by multiple factors such as the type and quantity of food consumed as well as the way it is cooked and prepared and what it is eaten with (e.g. a baked potato has a higher GI than the same potato eaten with butter). In addition,

the glycaemic response is also determined by an individual's glucose tolerance, which is defined as the body's ability to regulate blood glucose levels including its removal from the circulation. Typically, an individual with good glucose tolerance will display lower glycaemic responses to the same food or meal than someone with poor glucose tolerance (Rizkalla, Bellisle & Slama, 2002).

Figure 1.1: Examples of low and high glycaemic responses



Research has consistently shown that clinically impaired glucose tolerance, found in those with type 2 diabetes mellitus (T2DM), is associated with cognitive impairment in a variety of domains including verbal memory and speed of processing (Awad et al., 2004; Cukierman et al, 2005; Geijselaers et al, 2015; Messier, 2005). However, the association between glucose tolerance and cognition is not limited to just those with clinically impaired glucose tolerance. A review of the literature found that variance in glucose tolerance within normal levels can influence cognition, with poorer glucose regulators displaying cognitive impairments compared to good glucose regulators, even in healthy young adult samples (Lamport et al., 2009). Although, the reviewers stated there was no consistent method used across studies for determining good or poor glucose tolerance within the "healthy" range.

The four clinically defined categories of glucose tolerance, in order of increasing clinical impairment are; normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (NGT) and type 2 diabetes mellitus (T2DM). For the full clinical criteria for all four glucose tolerance categories as outlined by the World Health Organisation (2016), see Table 1.1.

Table 1.1: Diagnostic criteria (WHO, 2016) for normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes (T2DM).

Tolerance	Fasting Glucose Level	And/OR	2 Hour Glucose Level*
NGT	<6.1mmol/l	and	<7.8mmol/l
IFG	6.1 to 6.9mmol/l	and	<7.8mmol/l
IGT	<7.0mmol/l	and	≥7.8 and <11.1mmol/l
Diabetes (T2DM)	≥7.0mmol/l	or	≥11.1mmol/l

* Venous plasma glucose 2-h after ingestion of 75g oral glucose load

A recent global report released by the WHO (2016) stated that an estimated 422 million adults were living with diabetes (type 1 and 2) in 2014, compared to 108 million in 1980. It was reported that the global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5%. Along with cognitive impairments, diabetes has been consistently associated with reduced life expectancy, significant morbidity and reduced quality of life (WHO, 2006, 2016). Considering the increasing number of cases, research investigating the association between glycaemic response, glucose tolerance and cognition is important to advance understanding of how abnormalities in glucose regulation may affect the brain, and how nutritional interventions can potentially treat cognitive impairments in these populations.

1.3 Dietary manipulation, glycaemic response, and cognitive performance

The glycaemic response is largely affected by the quality and quantity of the CHO contained within a food or meal, as is glucose tolerance. The Glycaemic Index (GI) (Jenkins et al., 1981) is a classification system that assigns a value to CHO-containing foods, which indicates their rate of glucose release. A low GI food will result in a slow release of glucose into the blood, producing a lower glycaemic response. Conversely, a higher GI food results in a more rapid release of glucose into the blood, leading to a higher glycaemic response. Typically, foods containing complex CHOs (CHOs with longer saccharide chains i.e. polysaccharides) such as wholegrain foods and nuts, have lower GI values resulting in lower glycaemic responses. This is because complex CHOs are harder to break down than simple CHOs such as glucose, resulting in a longer period of digestion and a subsequently steadier rate of glucose release. The Glycaemic Load (GL) (Salmeron et al., 1997a, 1997b) is a classification system that assigns a value to a food or meal, which accounts for both its' GI and CHO quantity. Similarly, to GI, a lower GL food or meal typically produces a lower glycaemic response. Full definitions of GI and GL can be found in Chapter 2, Section 2.1.1.

Considering the relationship between GI, GL and glycaemic response, recent studies have investigated whether utilising these methods to manipulate the glycaemic response can also have an effect on cognitive performance in humans. Generally, (see Chapter 2 for a review) research examining healthy adults and children has reported better cognitive performance following a low GI/GL meal compared to a high GI/GL meal in various domains including memory, attention and speed of processing (Benton et al., 2003; Cooper et al., 2012, 2015;

Ingwersen et al., 2007; Lamport et al., 2014a, Micha et al., 2010; Nilsson et al., 2009, Young & Benton, 2014). With such positive findings in ostensibly healthy individuals with normal glucose tolerance, it is possible that even greater differences in cognitive performance following low and high GI foods could be seen in those with poor glucose tolerance such as those with T2DM. It is currently recommended that those with T2DM consume a low GI/GL diet to aid glycaemic control (ADA, 2008; Brand-Miller et al., 2003; Dyson et al., 2011; Evert et al., 2014). Surprisingly, research investigating GI/GL and cognitive performance in those with type 2 diabetes is limited and has returned mixed results (see chapter 2).

Although there is a wealth of research investigating GI/GL manipulation and cognition following consumption of a single meal. There are currently no studies that have extended this paradigm over three consequent meals in either a healthy or clinically impaired sample. It is possible that having multiple meals may magnify the effects of GI on cognition. In support, it is known that having multiple meals has cumulative effects on physiological responses such as glucose response. This well-established effect is known as the second meal effect (Wolever, 1988, 2006). It can be hypothesised that those with poorer glucose tolerance, such as patients with T2DM have the potential to have greater benefits from a low GI/GL diet than healthy individuals, due to overall poorer glycaemic control. This suggests that those with T2DM may also stand to gain more cognitive benefits by improving glycaemic control through the consumption of LGI/LGL diets.

Presently, it is clear that manipulating the GI/GL of multiple meals can affect the glycaemic response acutely, as well as glucose tolerance in the long term. However, the potential cognitive benefits that may be associated with diets varying in GI/GL remain largely unexplored beyond a single meal. The potential cognitive impact of GI/GL manipulation across multiple meals warrants investigation, especially in the light of recommendations that those with T2DM actively consume a low GI/GL diet (ADA, 2008; Evert et al., 2014).

1.4 The second meal effect and the second meal cognitive effect

As mentioned above, the ability of one meal to affect the glycaemic response to the following meal has been labelled the “second meal effect” (Wolever, 1988, 2006). Specifically, a lower glycaemic response to one meal is associated with a lower glycaemic response to the subsequent meal. Early evidence of the second meal effect demonstrated its’ presence over two subsequent meals during the day, typically breakfast followed by lunch (Jenkins et al., 1982; Liljeberg et al., 1999; Liljeberg & Bjorck, 2000). Several studies that have utilised GI as a method of manipulating the glycaemic response have consistently reported a lower glycaemic response at breakfast following a low GI evening meal compared to a high GI evening meal (Granfeldt., 2006; Lamport et al., 2011; Nilsson et al., 2006, 2008; Wolever, 1988). The findings from both sets of studies suggest that the second meal effect is present over the course of two subsequent meals, even if they are separated by an overnight fast. Furthermore, glycaemic effects can be seen beyond two eating episodes. For example, a review by Thomas and Elliot (2009) concluded that a low GI/GL diet lasting four weeks or longer can significantly improve measures of glucose tolerance such as glycated haemoglobin (HbA1c) levels.

The second meal effect should be regarded as an important part of designing and implementing cognitive research. Previously, a fasting period of two hours has been proposed as an acceptable duration to control for possible effects of previous dietary intake on cognitive function (e.g. Sunram-Lea et al., 2001). If it is accepted that there is a relationship between glucose levels and cognition, evidence of the second meal effect after an overnight fast suggests that substantially longer fasting periods than 2 hours are needed to control for prior nutritional intake when assessing cognition. It could be hypothesised that an earlier meal could affect cognitive performance after the next meal via a second meal effect. Only one study has investigated this hypothesis and found an HGI evening meal was associated with better verbal recall the following morning before and after a standardised HGI breakfast (Lamport et al., 2011). The HGI evening meal was also associated with better word recognition after but not before a standardised HGI breakfast. The researchers referred to this finding as the "second meal cognitive effect" (Lamport et al., 2011). However, this research has yet to be extended to beyond the breakfast period. For example, it is possible that the nature of breakfast and lunch may cumulatively affect glycaemic response and cognitive performance over the remainder of the day. This key question forms the basis of this thesis; specifically, how glycaemic response and cognitive function is affected following the consumption of multiple meals over one day.

It can be hypothesised that individuals with poorer glucose tolerance, such as those with T2DM stand to gain greater benefits from a low GI/GL diet than healthy individuals. The reason for this is that although a low GI/GL diet could also improve the glucose tolerance of healthy individuals, this improvement may be larger in T2DM due to the presence of poorer glycaemic control. This suggests that those with T2DM may also stand to gain more cognitive benefits by improving glycaemic control through the consumption of LGI/LGL diets.

1.5 Dietary manipulation, glucose tolerance and mood

The relationship between glycaemic response and subjective mood is also explored throughout this thesis. Measuring subjective mood is important as the cognitive tasks implemented here (see Chapter 3) monitor task performance, but do not measure subjective mood outcomes which can provide further information about an individuals' cognition and how it differs between glycaemic conditions. For example, an individual may not display significantly different accuracy scores on a task between conditions but may be significantly less alert during one condition. Therefore, these subjective mood measures are worthy of investigation alongside the cognitive performance tasks. Whilst the current literature supports the association between meal consumption and improved mood compared to meal omission in healthy samples, there is a limited number of studies that implement the concept of GI/GL (see Chapter 2 for a full review). One study that investigated the acute effects of GI on cognition in a healthy sample found that school children reported significantly higher levels of happiness and alertness following a low GI breakfast compared to a high GI alternative (Micha et al., 2011). However, the current literature exploring the association between glycaemic response and subjective mood in acute settings is largely limited to a single meal testing paradigm, with focus on the breakfast meal (see Chapter 2). Clearly, there is a distinct lack of

research that has investigated the relationship between glycaemic response and subjective mood throughout the day, using a multiple-meal paradigm and the GI/GL concept. This is more surprising when one considers that longitudinal research has indicated an association between progressively higher dietary GI/GL and worsening mood outcome measures (Breymeyer et al., 2016; Cheatham et al., 2009; Gangwisch et al., 2015). This suggests that the consumption of a LGI/LGL diet may have measurably different cumulative effects on subjective mood measures throughout a test day when compared to a HGI/HGL diet. The lack of research investigating glycaemic response and mood across multiple GI/GL meals throughout the day results in a knowledge gap within the literature and provides a clear rationale for future research to examine this area.

As aforementioned, previous research has indicated that T2DM is associated with cognitive impairments in multiple domains such as verbal memory and speed of processing (Awad et al., 2004; Cukierman et al, 2005; Geijselaers et al, 2015; Messier, 2005). It has also been reported that poor glucose regulators display more cognitive impairments compared to good glucose regulators, even when the entire sample is within NGT levels (Lamport et al., 2009). Taken together, these findings suggest that poorer glucose tolerance is associated with increasing cognitive deficits. Given this association, it is also plausible that subjective mood may be negatively affected by a poorer glucose tolerance status. Indeed, the relationship between T2DM and increased depression rates is well-established, although beyond the scope of the present thesis (Ascher-Svanum et al., 2015; Gross et al., 2005; Gonzalez et al., 2015; Lustman et al., 2000; Maraldi et al., 2007; Papelbaum et al., 2011; Van der Does et al., 1996, 1998; Zhang et al., 2015). Whilst there is a wealth of longitudinal research linking T2DM with clinical mood disorders, there is a distinct lack of acute multi-meal GI/GL studies that explore the association between glycaemic response and subjective mood in this clinical group. Furthermore, it has also been reported that participants with better glucose tolerance within an ostensibly healthy sample display better subjective mood than those with poorer glucose tolerance within the NGT range (Nabb & Benton, 2006). This finding suggests that measurable differences in subjective mood outcomes can be detected within a healthy sample and are not limited to differences between clinical groups.

1.6 Aims, objectives and hypothesis of the thesis

Collectively, the series of studies presented within this thesis aimed to investigate the relationship between the glycaemic response, cognitive performance and mood specifically within the context of a multi-meal paradigm across one day. In addition, this relationship is considered in populations with different levels of glucose tolerance, e.g. healthy adults and adults with type 2 diabetes. The concept of GI was utilised to create two different meal profile conditions (Favourable Glycaemic Profile vs. Unfavourable Glycaemic Profile). Cognitive performance and mood were assessed as the key outcome variables. Specific aims and research questions are detailed below.

1.6.1 Primary aims

1.6.1.1 To design two novel meal profiles that produce different glycaemic response profiles, utilising the concept of GI (Study 1)

The first study was a randomised cross-over dietary intervention study that investigated whether two meal profiles varying in GI can produce significantly different glycaemic response profiles across the day. Given that the majority of previous cognitive research utilising GI manipulation has focussed on a single meal, typically the breakfast meal, it was necessary to design two novel meal profiles to investigate this aim. The two meal profiles contained a breakfast, lunch and afternoon snack, which mimic usual eating patterns, and utilised the concept of GI so that one meal profile contained only low GI mixed meals (Favourable Glycaemic Profile condition), whereas the other only contained high GI mixed meals (Unfavourable Glycaemic Profile condition). Both diets were isocaloric and matched for macronutrients to avoid confounding effects. It was initially important to design two meal profiles which produced measurably different glycaemic response profiles across the day, so that these could then be applied to the investigation of cognitive effects in the following studies.

1.6.1.2 To investigate the relationship between cognitive performance and glycaemic response over the course of three consecutive meals (Study 2)

The second study was a randomised, controlled cross-over study that investigated cognitive performance following the consumption of the two meal profiles designed in study one (FGP vs. UGP). Previous research has suggested a relationship between glycaemic response and cognitive performance with a single meal testing paradigm, with evidence generally favouring low-GI meal consumption (Philippou & Constantinou, 2014). Study two extended this testing paradigm from a single meal to three consecutive meals, which mimic usual eating patterns, to investigate the relationship between glycaemic response and cognitive performance across the day.

1.6.1.3 To investigate the relationship between cognitive performance and glycaemic response over the course of three consecutive meals in those with type 2 diabetes (Study 3)

The third study employed the same cross-over design and meal profiles as the previous two studies to investigate the relationship between glycaemic response and cognitive performance in T2DM. There are well established cognitive impairments in populations with T2DM (Awad et al., 2004). These cognitive impairments could potentially be attenuated by improving glycaemic response acutely. By adopting the two meal profiles designed to produce opposing glycaemic response profiles, this study investigates glycaemic response and cognitive performance across the day in T2DM.

1.6.2 Secondary aims

1.6.2.1 To investigate the relationship between mood and glycaemic response over the course of three consecutive meals (Study 1, 2 & 3)

There is some evidence that acute low GI meal consumption is associated with a better mood state in ostensibly healthy individuals (Micha et al., 2011), however this has not been investigated within the context of multiple meals across one day. It is also possible that those with greater glycaemic variability such as those with T2DM will be more sensitive to the meal manipulations. Therefore, effects on mood will be assessed in both healthy adults and adults with T2DM.

1.6.2.2 To investigate if glucose tolerance status within a sample predicts cognitive performance and subjective mood outcomes in a multi-meal paradigm (Study 2 & 3)

As previously mentioned, the literature supports a relationship between glucose tolerance and cognition, with poorer cognitive performance and mood often seen in those with poorer glucose tolerance. To investigate this relationship in the present studies, a glucose composite score was implemented to split each study sample into good and poor glucose regulators (see Chapter 3 for calculation method). Regulator Type was then included in Linear Mixed Model analyses as a covariate to measure its' viability as a predictor of cognitive performance and mood.

1.6.4 Hypotheses

1.6.4.1 The meal profiles and glycaemic response

The consumption of low GI meals (FGP condition) will be associated with an improved postprandial glycaemic response profile compared to the consumption of high GI meals (UGP condition) in both clinically healthy and T2DM individuals. Specifically, the FGP condition will be associated with a lower and more stable glycaemic response profile (less pronounced and less frequent peaks and troughs) across the day compared to the UGP condition.

1.6.4.2 The relationship between cognitive performance and glycaemic response

A more stable and lower glycaemic response will be associated with better cognitive performance across the day. It has been hypothesised that the FGP condition will be associated with an improved postprandial glycaemic response profile compared to the UGP condition in healthy adults and adults with T2DM (See Section 1.6.4.1). Therefore, it is hypothesised that the FGP condition will be associated with better cognitive performance across the day compared to the UGP condition in both clinically healthy and T2DM individuals.

1.6.4.3 The relationship between subjective mood and glycaemic response

A more stable and lower glycaemic response will be associated with better subjective mood across the day. It has been hypothesised that the FGP condition will be associated with an improved postprandial glycaemic response profile compared to the UGP condition in healthy adults and adults with T2DM (See Section 1.6.4.1). Therefore, it is hypothesised that the FGP condition will be associated with better subjective mood across the day compared to the UGP condition in both clinically healthy and T2DM individuals.

1.6.4.4 The relationship between glucose tolerance, cognitive performance and subjective mood

Previous research has indicated that individuals with poorer glucose tolerance often display poorer cognitive performance and subjective mood when compared to those with better glucose tolerance within the same sample (See Chapter 2). Therefore, it is hypothesised that those labelled as comparatively poorer glucose regulators within the clinically healthy and T2DM samples (through the use of a glucose composite score) will display poorer cognitive performance and subjective mood across the day when compared to the better glucose regulators.

Chapter 2

The Relationship Between Glycaemic Response, Glucose Tolerance, Cognitive Performance and Mood

This chapter consists of a comprehensive systematic literature review examining the relationship between glycaemic response, glucose tolerance, cognitive performance and mood. This includes a review of the individual factors (e.g. glucose tolerance) and dietary interventions (GI and GL) that can influence the glycaemic response profile and the interaction between these factors when considering cognitive performance and mood outcomes. Finally, underlying mechanisms that could explain the relationship between glycaemic response, glucose tolerance, cognitive performance and mood are discussed.

2.1 Defining glycaemic response

The glycaemic response can be defined as the postprandial blood glucose response elicited when a CHO-containing food or meal is ingested (Augustin et al., 2015). The glycaemic response to a food or meal can be categorised as either a "low" or "high" glycaemic response. A low glycaemic response can be described as a slow and steady rise in blood glucose concentration to a low and prolonged peak, followed by a steady decrease back to fasting levels. Conversely, a high glycaemic response can be described as a rapid increase in blood glucose concentrations to a high and sharp peak, followed by a fast decline, returning to and occasionally below fasting levels before returning to normal (see Figure 1.1, Chapter 1). The glycaemic response to any given food or meal is determined by a combination of multiple factors, which are discussed below.

2.1.1 Factors affecting glycaemic response

2.1.1.1 Glycaemic Index

Conceptually, the GI is a measure of carbohydrate "quality" within a food or meal (Jenkins et al., 1981). The GI is the glycaemic response to a food containing 50g of available carbohydrate, expressed as a percentage (0-100) of the glycaemic response elicited by 50g of reference carbohydrate i.e. anhydrous glucose or white bread (ISO, 2010). For clarity, to calculate the GI of a food, an individual must consume a portion of food containing exactly 50g of available carbohydrate. Their glycaemic response would then be compared to the glycaemic response produced by consuming exactly 50g of the reference carbohydrate (glucose or white bread). The resulting area under the curve shared between the two glycaemic responses, expressed as a percentage, is the GI value of the food.

The GI values of many foods and products have been calculated and published in international tables (Foster-Powell et al., 1995, 2002; Henry et al., 2005; Atkinson et al., 2008). There are currently three categories in which a food can be placed according to its GI%. These are low (≤55), medium (56-69), and high (≥ 70). The GI of a food or meal indicates the rate at which

the available carbohydrate will be digested, and the resulting glucose will enter the bloodstream. A high GI food will typically contain carbohydrate that is digested, absorbed and metabolised at a faster rate than a low GI food. Therefore, the GI (i.e. carbohydrate quality) of a food can affect the shape of the glycaemic response, with high GI foods associated with a high glycaemic response.

The clinical utility of GI if applied to mixed meals was challenged by Coulston et al. (1984), who reported glucose responses to test meals were totally disparate from what would have been expected from published GI values of the meal components. In response, Wolever & Jenkins (1986) highlighted that these findings were based on an inappropriate assessment of the data and demonstrated that the concept of GI is not limited to single foods, as it can be applied to mixed meals. Although the GI of a single food can be measured with precision, the GI of a mixed meal is obtained through calculation of the GI values and CHO contents of its' comprising foods. Using the method laid out by Wolever & Jenkins (1986), one would first calculate the total CHO of the meal, then work out the percentage of total CHO provided by each food and multiply each foods' value by its' GI value. The total sum of these values is the GI of the mixed meal. In relation to glycaemic response, a low GI meal is associated a lower glycaemic response in the same individual than a high GI meal (see Table 2.1 for an example taken from the breakfast meals used throughout this thesis).

Table 2.1: Examples of low and high GI breakfast meals.

Condition		GI	Weight (g)	CHO (g)	PCF (%)	PGI (GI*PCF/100)
LGI Breakfast	All Bran Cereal	44	29	13.92	26.5	11.7
	Skimmed Milk	48	126	6.3	12	5.8
	Apple Juice	40	226	26.44	50.3	20.1
	Yoghurt	35	84	5.88	11.2	3.9
	Meal Total	*N/A*	*465*	*52.54*	*100*	*41.4*
HGI Breakfast	Corn Flakes	93	30	25.2	47.3	44
	Skimmed Milk	48	220	11	20.6	9.9
	White Bread	75	38	16.95	31.8	23.9
	Flora Spread	0	3	0.02	0.3	0
	Meal Total	*N/A*	*291*	*53.35*	*100*	*77.8*

*CHO = Carbohydrate content, GI = Glycaemic Index, PCF = proportion of CHO from each food, PGI = Portion GI.

Although GI and the glycaemic response are similar concepts, it is important to note that they are not the same. The GI is a fixed measure of CHO content quality of a food, which can be measured consistently under strict methodological conditions in the same individual (Wolever et al., 1990). The glycaemic response is an individual's blood glucose reaction to the consumption of food. The key difference is that the GI of a food should be constant if measured correctly, whereas the glycaemic response can be influenced by a multitude of factors such as individual glucose tolerance and quantity of food. In summary, whilst the GI of a food or meal can be used to predict whether a low or high glycaemic response will likely

follow consumption, it is important to realise that other factors contribute to the glycaemic response.

2.1.1.2 Glycaemic Load

As mentioned above, a food's GI represents the quality of CHO that it contains. However, the concept of GI fails to consider the quantity of CHO that a food serving contains. As the quantity of CHO also has an effect on the glycaemic response elicited from consumption, glycaemic load (GL) was introduced by Salmeron et al. (1997, a, b). The concept of GL is to quantify the glycaemic potential of a food by accounting for both quality and quantity of CHO. To calculate the GL of an individual food, one would multiply its' GI by the amount of CHO (g), and then divide by 100 (Brand-Miller et al., 2003; Foster-Powell et al., 2002). Therefore, a GL value of a food represents both the amount of CHO it contains and its' GI. Foods with a GL ≤10 have been classified as low GL, and those with a value ≥20 as high GL (Barclay, Brand-Miller & Wolever, 2005). An important distinction between GI and GL needs to be made clear. The above equation shows us that either a low-GI/high-CHO food or a high-GI/low-CHO food can have the same GL. Research suggests that two such foods will have similar effects on postprandial glycaemia but will have very different metabolic effects (Ball et al., 2003; Wolever & Mehling, 2003). For example, the GL of a meal can be reduced by either reducing CHO content or by reducing the dietary GI (Wolever & Mehling, 2003). Whilst both manoeuvres will reduce the acute glycaemic and insulin response (Chew et al., 1988; Indar-Brown et al., 1992), they have different effects on postprandial plasma free fatty acids (FFAs). Specifically, research has shown that replacing CHO content with isoenergetic amounts of monounsaturated fatty acids increases postprandial FFA levels in both healthy and T2DM samples (Wolever, Bentum-Williams, Jenkins, 1995; Wolever et al., 1992). Long-term reductions in dietary CHO content have been shown to raise postprandial FFA concentrations by >30% in T2DM samples (Tsihlias et al., 2000). Conversely, a reduction in meal GI with maintenance of CHO content reduces postprandial FFA concentrations (Wolever, Bentum-Williams, Jenkins, 1995). Previous research has indicated an association between elevated FFA levels and reduction in insulin secretion and action (Boden et al., 1994; Carpentier et al., 1999; Zhou & Grill, 1994), increasing the risk of developing T2DM (Ferrannini, 1998; Gerich, 1998; Paolisso et al., 1995). Considering the link between increased insulin resistance and poorer cognitive performance (see Section 2.4), this provides evidence that two meals with the same GL may have different effects on cognition dependent upon their CHO and GI content.

For clarity, there are two slightly different ways to calculate dietary GL. It is important to note that there is no current consensus on which method should be applied, but the determining factor appears to be length of study. Specifically, studies that extend testing beyond a single meal (e.g. over several days) will typically adjust the diet's GL for total energy intake (e.g. Ebbeling et al., 2003; Levitan et al., 2007; Salmeron et al., 1997b; Wolever & Mehling, 2003). Whereas acute studies generally do not adjust for energy intake and instead apply the Foster-Powell et al. (2002) method, where the GL for the day's diet is calculated as the sum of each meal's GL. Therefore, it is important that a study indicates which method of GL calculation is employed as applying these two different methods to a data set involving a diet rather than a

single meal can return significant differences in the results produced and their interpretation (Wolever, 2006; Wolever & Mehling, 2003). For example, Wolever (2006) used both methods to calculate the GL of a LGI diet that was implemented in a study by Wolever and Mehling (2003). Interestingly, there was a significant drop in GL consumption between the start and end of the trial when not adjusting GL for energy intake. However, when GL was adjusted for energy intake, there was a slight (but non-significant) increase in dietary GL.

Similar to GI, research has shown that foods with a higher GL are associated with higher glycaemic responses (Lee & Wolever, 1998; Wolever, 2006; Wolever & Bolognesi, 1996). In healthy individuals, stepwise increases in GL have been found to predict stepwise elevations in postprandial blood glucose levels (Brand-Miller et al., 2003). Typically, there is a levelling off of glycaemic response at the higher doses of GL. To conclude, the concept of GL can be applied in a similar way to GI. If an individual consumes a high GL food, they would be expected to produce a higher glycaemic response than they would after consuming a low GL food. The series of studies throughout this thesis calculate and report both dietary GI and GL for all test meals. Since all studies were acute investigations with energy and macronutrients matched between meals, the Foster-Powell et al. (2002) was implemented and GL was not adjusted for energy intake.

2.1.1.3 Glucose Tolerance

Glucose tolerance can be defined as the body's ability to regulate blood glucose levels. The terms glucose tolerance and glucose regulation are interchangeable terms, but only glucose tolerance will be used throughout this thesis. As shown in Table 1.1 (Chapter 1), there are currently four categories of glucose tolerance; normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (T2DM). These categories and their diagnostic criteria are defined by the World Health Organization (2016; see Chapter 1, Table 1.1), with each category representing a poorer level of glucose tolerance than the last. It is important to note that other types of diabetes such as type 1 (total or near total loss of insulin producing pancreas cells) do exist. However, the focus of this thesis is NGT and T2DM as they represent the extreme ends of the glucose tolerance continuum. NGT can be defined as the absence of abnormality in glucose tolerance, whereas T2DM is the resulting condition of worsening glucose tolerance characterised by increasing insulin resistance and reduced insulin production in the pancreas.

The current diagnostic procedure for glucose tolerance is formed by two tests; a fasting glucose measurement and an oral glucose tolerance test (OGTT). Both tests are required for accurately diagnosing NGT, IFG and IGT. When diagnosing T2DM, the World Health Organisation recommends that either a fasting glucose reading or the OGTT is used (WHO, 2016). The fasting glucose test is carried out by taking a simple venous blood sample. The OGTT requires the individual to consume a 75g glucose load after a minimum eight hour fast (WHO, 1999). Glucose concentrations two hours after glucose administration are used for diagnosis of glucose tolerance (See Table 1.1, Chapter 1). The diagnostic thresholds of glucose concentrations have been selected on the basis of their association with increased negative health outcomes such as cardiovascular problems (WHO, 2006). The measuring of glycated

haemoglobin levels (HbA1c) has also been suggested as a diagnostic tool by the International Expert Committee (IEC, 2009). An HbA1c reading reflects average plasma glucose levels over the previous eight to twelve weeks (Nathan et al., 2007). The IEC recommended that a reading of 6.5% or higher is enough to diagnose T2DM. The relationship between HbA1c and microvascular complications was later reviewed by the WHO, who concluded that HbA1c can be used as a diagnostic test for T2DM (HbA1c ≥ 6.5%) providing that stringent quality assurance tests are in place, and that no conditions are present which could affect accurate measurement (WHO, 2016). WHO also recommended that a value below 6.5% did not exclude T2DM diagnosed using standard glucose tests. Finally, the consultation stated that there was insufficient evidence to make any formal recommendation on the interpretation of HbA1c levels under 6.5%. Interestingly, cognitive research has reported an association between higher HbA1c readings and poorer mood outcomes in T2DM samples, but these studies tend to utilise longitudinal or cross-sectional research designs (see Section 2.3). In acute studies assessing cognitive performance and mood in T2DM samples, the use of HbA1c readings is rarer with researchers splitting good and bad glucose regulators through other methods such as fasting glucose levels (see Section 2.2-2.3). However, in this thesis a glucose composite score was employed (see Chapter 3 for details) as this has been suggested to have more ecological validity than the use of a single glycaemic parameter in acute settings (Lamport et al., 2009).

Similar to the glycaemic response, an individual's glucose tolerance is not a constant value which can be measured reproducibly. Glucose tolerance is affected by a wide range of factors such as an individual's age, gender, ethnicity, and weight. Whilst this is not an exhaustive list of potential factors, it serves to illustrate how variable glucose tolerance can be between individuals and even within individuals on a day by day basis. Research has shown that the aging process has a deleterious effect on glucose tolerance, with poorer glucose tolerance found in older aged individuals (Basu et al., 2003; DeFronzo, 1981; Shimokata, 1991). When comparing males and females, it has been suggested that greater amounts of visceral and hepatic adipose tissue, in conjunction with the lack of a possible protective effect of oestrogen may explain higher insulin resistance found in men compared with women (Geer & Shen, 2009). Although literature that examines the link between ethnicity and glucose tolerance can vary depending on the ethnicities included and location of the testing, regional data suggests that areas such as the Middle East and North Africa are more likely to have a higher T2DM rate (Spanakis, 2013). Interestingly, overweight individuals have a greater risk of developing T2DM (Colditz et al., 1995; Hamman et al., 2006; Wannamethee & Shaper, 1999). Severe obesity has been found to be a predictor of poor glucose tolerance in children, irrespective of ethnic group (Sinha et al., 2002). Furthermore, obesity has been shown to share an association with cognitive impairment independently of T2DM (Farr et al., 2008; Miller & Spencer, 2014). This is thought to be occur through similar underlying mechanisms that associate T2DM with cognitive impairment such as neuroinflammation (see Section 2.4). It is important to note that there are many factors that can affect glucose tolerance, which work in tandem with one another. Therefore, an individual's glucose tolerance is highly variable and cannot be easily predicted by a single factor.

In relation to glycaemic response, research suggests that two individuals varying in glucose tolerance will produce measurably different glycaemic responses when consuming the same

food (Bantle et al., 1983). Generally, those with poorer glucose tolerance will begin at a higher fasting glucose value, reach a higher level of glucose concentration and will take a longer time to return to fasting levels after food consumption. This results in an excessive and prolonged blood glucose peak, which is a major health problem in those with T2DM, putting them at increased risk of vascular damage (Nnadi, 2016). As a result, it has been recommended that those with T2DM look to apply the glycaemic index to their diet to assist glucose control (Hodge et al., 2004).

In summary, an individual's glucose tolerance can help to predict whether a low or high glycaemic response is likely to occur following food consumption. Specifically, an individual with poorer glucose tolerance would be expected to produce a higher glycaemic response to the same food as an individual with good glucose tolerance. It is important to acknowledge glucose tolerance is determined by the combination of a multiple of factors, such as age and ethnicity, which work in tandem with one another. Research suggests that glucose tolerance can be improved by applying dietary interventions such as the glycaemic index as well as lifestyle changes such as better management of weight (Dengel et al., 1998; Willet, Manson & Liu, 2002). Therefore, by improving an individual's glucose tolerance it can be expected that they would display lower glycaemic responses postprandially.

2.1.1.4 Second meal and long-term diet effects

Research has shown the presence of a 'second-meal effect' which can be defined as the glycaemic response of one meal having an influence on the glycaemic response to a subsequent meal, even after an overnight fast (Wolever et al., 1988). Specifically, consuming an earlier low GI meal is expected to reduce the glycaemic response shown at the following meal. A review of the literature concluded that researchers who conduct glycaemic testing should understand the potential impact of the second meal effect, and that failure to control for previous meal consumption will have profound effects on results (Fletcher et al., 2012). Research investigating the link between glycaemic response and cognitive performance has suggested that a second meal cognitive effect could exist, with the GI of an evening meal influencing cognitive performance the following day after breakfast (Lamport et al., 2011). The researchers reported that the consumption of a high GI evening meal was associated with better word recognition the following morning, after a standardised high GI breakfast, when compared with a low GI evening meal. This suggests that an overnight fast may not be sufficient to control for previous consumption when considering cognitive outcomes.

The glycaemic effects of previous nutritional consumption is not solely limited to the one meal before a test meal. There is a wealth of literature showing that the habitual diet of an individual can greatly affect their glucose tolerance, which in turn influences their glycaemic response to a particular test meal. Research has consistently found that the consumption of a high GI/GL diet is associated with an increased risk of developing T2DM (Bhupathiraju et al., 2014; Dong et al., 2011; Schulze et al., 2004; Villegas et al., 2007). However, the implementation of a low GI diet has reliably improved glucose tolerance in multiple samples with T2DM (Jarvi et al., 1999; Wolever et al., 1992). The consumption of low GI diets has been recommended in evidence-based nutrition guidelines by several groups including Diabetes UK

(Dyson et al., 2011) and the Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes (Mann et al., 2004; Krane et al., 2007). Both groups suggested that the implementation of a low GI diet can help manage and prevent T2DM by improving glycaemic control.

To conclude, previous nutritional consumption in both the short and long term can influence the glycaemic response seen when an individual consumes a food. The immediate impact of the GI of one meal can be seen on the glycaemic response to the subsequent meal, with a lower GI typically producing a lower glycaemic response. This second meal effect can even occur after an overnight fast and may also have cognitive consequences. Evidence also suggests that the habitual diet of an individual will affect their glucose tolerance, with a lower GI diet leading to improved glycaemic control. This means that an individual who displayed a high glycaemic response postprandially would display a lower glycaemic response to the same food if they implemented a low GI diet.

2.2 A systematic review of glycaemic response, glucose tolerance and cognitive performance

2.2.1 Objectives

The review conducted here aims to assess the current relationship between glycaemic response and cognitive performance by compiling the findings in the current literature. By doing so, this review aims to identify whether a LGI or HGI meal condition is associated with an improved glycaemic response, and whether such a response is associated with comparatively better cognitive performance outcomes. In addition, the underlying effect of glucose tolerance will be considered by investigating whether glycaemic response and cognitive performance differences occurred within studies that compared good and poor glucose regulators.

2.2.2 Methods

2.2.2.1 Protocol and Registration

The present systematic review was conducted solely within the process of this PhD thesis and is, therefore, not registered on literature review databases such as Prospero. Thus, a review procotol can not be publically accessed via a web address. Therefore, the methods and protocol implemented is described below.

2.2.2.2 Eligibility criteria

Research were deemed eligible for review if the following criteria were met; *1*) comparisons between low GI and high GI foods or drinks, or between low GL and high GL foods or drinks, *2*) studies in which GI/GL was either estimated or measured, 3) studies which measure cognitive performance following the consumption of test foods or drinks, *4*) studies conducted in humans (all ages), *5*) studies published in English. The exclusion criteria were as follows: *1*)

review articles, *2*) studies where the effect of GI or GL on cognitive function was not the main outcome measure, *3*) studies where no comparison between low and GI/GL foods is made, *4*) studies in which the same food/drink was consumed at different rates to simulate low and high GI/GL conditions, *5*) conference abstracts.

2.2.2.3 Information sources

The following databases were searched in order to find eligible research to review; *1*) Google Scholar, *2*) Medline (Pubmed), *3*) Web of Science, *4*) PsycINFO, *5*) Cochrane Register of Controlled Trials (CENTRAL). All databases were repeatedly accessed and searched by the reviewer from March 2016 to September 2019.

2.2.2.4 Search and Study selection

A search of all information sources was performed using the keywords glycaemic index, glycaemic load, attention, memory, cognition and cognitive performance (including truncated and Americanised forms). Once a search had been conducted within a database, the title and abstracts of each piece of literature were examined in order to eliminate any that were deemed not relevant upon first screening. After this initial process, a further examination of each study was conducted to check eligibility according to the inclusion/exclusion criteria. The complete process resulted in eighteen studies being deemed eligible for review. Relevant studies cited in articles identified through the initial search were also considered. All research included had been subject to peer/editorial review, which led to conference abstracts being omitted from the present review.

2.2.2.5 Data items and data collection process

The primary data items were glycaemic response and cognitive performance outcomes. The method in which the glycaemic response was measured was not limited to a single method. For example, studies were included whether they used capillary finger prick or continuous glucose monitoring methods to measure glycaemic response. Cognitive data was considered for all domains that were measured, rather than focusing on a single cognitive domain in the current review. Data extraction from eligible studies was performed by the reviewer alone, and studies were not included if data was not clearly provided by the researchers. The data extracted from each study were as follows: *1*) sample characteristics, *2*) the design of the study, *3*) how the carbohydrate source was manipulated, *4*) the GI and GL values of the test foods, *5*) blood glucose sampling times (if conducted), *6*) the cognitive functions tests used, *7*) the cognitive domains assessed, *8*) the times that cognitive tests were administered, and *9*) the findings and timings of any cognitive benefits observed.

2.2.2.6 Summary measures

The principal summary measures included a significant difference in means, where a significant benefit of a LGI or HGI food/drink could be identified for glycaemic or cognitive outcome measures.

2.2.2.7 Synthesis of results

In order to produce a clear synthesis of results across the reviewed research, a summary table (see Table 2.2) was created to the data extraction from each study along with the findings (e.g. cognitive domains tested and benefits of each condition). In addition, Table 2.3 was also created in order to identify which cognitive domains were most sensitive to glycaemic interventions across studies. These findings are discussed in detail below (see Section 2.2.2).

2.2.3 Results

2.2.3.1 Study selection

An initial search of the literature returned a total of 94 studies, which were then screened and assessed for eligibility. Through this process, 76 studies were deemed ineligible for review leaving a total of 18 studies which were eligible. This process is presented below in Figure 2.1.

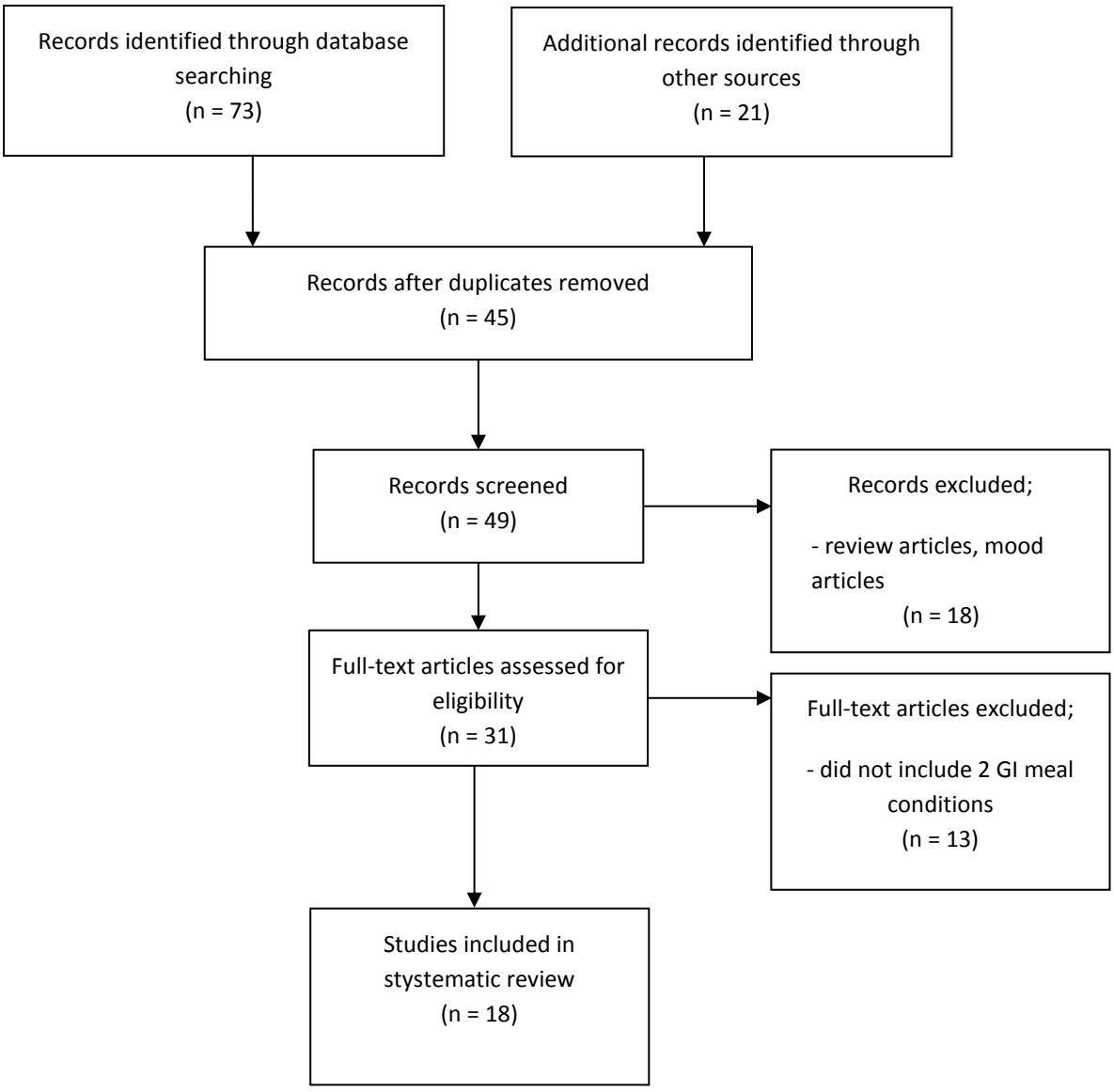


Figure 2.1: The study selection process stages for the systematic review.

2.2.3.2 Studies investigating the relationship between glycaemic response, glucose tolerance and cognitive performance

Out of the eighteen eligible studies, seventeen studies were acute investigations of cognitive performance across the morning following the consumption of test meals that varied in GI/GL. The one exception to this was Lamport et al. (2011) who implemented GI/GL conditions in the evening and then carried out cognitive testing the following morning after the consumption of a standardised high GI/GL breakfast. Taken together, these studies generally favour the consumption of LGI/LGL meals for cognitive benefits but results were inconsistent (see Table 2.2 for a summary). To summarise, eight studies reported cognitive improvements following a LGI/LGL meal (Benton et al., 2003; Cooper et al., 2012, 2015; Ingwersen et al., 2007; Lamport et al., 2014a; Mahoney et al., 2005; Nilsson et al., 2012; Papanikolaou et al., 2006), three

found a HGI/HGL meal to be beneficial (Lamport et al., 2011; Micha et al., 2010; Smith & Foster, 2008), five reported no differences between GI/GL conditions (Benton et al., 2007; Brindal et al., 2012; Kaplan et al., 2000; Lamport et al., 2013, 2014b) whilst two studies reported benefits following both LGI/LGL and HGI/HGL meals (Micha et al., 2011; Young & Benton, 2014).

A likely factor contributing to the mixed findings is the age range of the participants being investigated. It is known that a child's brain is relatively bigger and more active per unit weight compared with the brain of an adult, resulting in a higher utilization of glucose (Chugani, 1998). The increased activity of the child's brain means that they could be more susceptible to the provision of glucose. In the context of the reviewed studies, it may be possible that children respond to the mere presence of exogenous glucose rather than the type necessarily. Furthermore, this may also explain why more immediate benefits are seen following a HGI/HGL meal and more later postprandial benefits are reported following a LGI/LGL meal. For example, a slower release of glucose occurring after LGI/LGL meal consumption would mean a higher level of glucose availability for the brain in the later postprandial phase compared to a HGI/HGL meal, whereas the opposite would be true in the earlier postprandial phase. Indeed, previous research has indicated that an exaggerated insulin response, often occurring after HGI meal consumption, can lead to a period of reactive hypoglycaemia (Brun, Fedou & Mercier, 2000). Therefore, lower glucose availability to the brain could be reasonably expected in the later postprandial phase when a comparatively higher GI meal has been consumed.

Development through the adolescence period, defined by the WHO as an age range of ten to nineteen (WHO, 2016), is another age related factor which could be contributing to mixed results. Previous research has indicated that basal insulin secretion increases during puberty before returning to pre-pubertal levels during adulthood (Caprio et al., 1989). It has also been shown that fasting glucose levels remain constant during puberty, which suggests an increase in insulin resistance (Savage et al., 1992). Research investigating this has suggested that the changes in insulin secretion and sensitivity are linked to increases in growth hormone secretion, which reach a life-time peak during puberty before decreasing by the age of 21 (Berneis & Keller, 1996; Jorgensen et al., 1995; Kyho, O'Sullivan & Hoffman, 1996; Savine & Sonksen, 2000). Previous research has also indicated that the aging process across the adult lifespan is associated with poorer glucose tolerance and the dysregulation of associated neuroendocrine processes that can affect cognitive function (Awad, 2002; Messier, 2004; Gold et al., 2005). The combination of poorer glucose tolerance and cognitive deficits with increasing age suggests that older participants may be particularly sensitive to glucose facilitation effects. For example, improvements in episodic memory have frequently been observed in elderly participants following glucose ingestion (Hall et al., 1989; Manning et al., 1992; Parsons and Gold, 1992; Riby et al., 2006; Smith et al., 2011). Interestingly, Meikle et al. (2004) reported that task difficulty moderated the facilitative effects of glucose on episodic memory in the middle-aged. This suggests that older participants may be more sensitive to glucose administration due to age-related cognitive decline, which may be more prominent with increased task demand. Given the large age range across the eighteen reviewed studies (6-82 years) it is likely that insulin secretion and sensitivity are different across the age spectrum of participants, which could affect cognitive functioning, contributing to the mixed results seen across studies.

There are also a number of methodological discrepancies between studies that could also explain the mixed findings across the reviewed research. Firstly, only four studies succeeded in matching both energy and macronutrient content between conditions (Cooper et al., 2012, 2015; Lamport et al., 2011; Young & Benton, 2014), three matched calories and carbohydrate content (Lamport et al., 2013, 2014a, 2014b), one only matched for carbohydrate content (Papanikolaou et al., 2006), whilst the remaining ten studies did not match either energy or macronutrient content (Benton et al., 2003, 2007; Brindal et al., 2012; Ingwersen et al., 2007; Kaplan et al., 2000; Mahoney et al., 2005; Micha et al., 2010, 2011; Nilsson et al., 2012; Smith & Foster, 2008). Previous research has indicated that the dose of glucose moderates the glucose facilitation effect (Gold, 1986; Riby, Meikle & Glover, 2004), whilst fat and protein have been reported to share a negative relationship with postprandial glycaemia (Jenkins et al., 1981). Animal models have supported this by indicating that insulin responses to glucose in rats were augmented 3-fold and 1.5-fold by the addition of whey protein or oleic acid respectively (Gunnarsson et al., 2006). In human samples, a meal with additional protein has been associated with an augmented insulin response (Peters & Davidson, 1993; Nuttall et al., 1984), whilst additional fat content has been associated with attenuated postprandial rises in glucose (Collier & O'Dea, 1983; Gentilcore et al., 2006). Considering the majority of the reviewed studies did not match these aspects between two meals, it is likely that the difference in energy and macronutrient content is having a confounding effect on glycaemic and cognitive performance outcomes, making attribution of results to GI/GL variation between conditions more difficult.

The method, frequency and assessment times that blood glucose levels were measured varied greatly across studies, with four studies not taking glucose measurements (Benton et al., 2007; Cooper et al., 2012; Ingwersen et al., 2007; Mahoney et al., 2005). Of the fourteen studies that did measure blood glucose levels, thirteen implemented the use of capillary finger pricks, whereas only one employed a continuous glucose monitoring system (CGM) (Brindal et al., 2012). The frequency that the glycaemic response was assessed via capillary finger pricks ranged from two times (Micha et al., 2010, 2011) to eight times (Lamport et al., 2013, 2014a, 2014b; Nilsson et al., 2012) whilst Brindal et al. (2012) measured blood glucose forty-one times due to the advantage of the CGM. Surprisingly, the length of time blood glucose was measured also varied greatly across studies with the last measurement of glucose being recorded in a range of seventy-five to two-hundred and thirty minutes post meal consumption. The large differences in the frequency and time period of glycaemic testing may occur because the focus of the reviewed studies is usually the examination of cognitive differences between GI/GL conditions post consumption, with less emphasis being placed on tracking differences in the glycaemic responses that are produced. There is a need for appropriate glycaemic measurements in future research as it is plausible that significant differences in cognitive performance may be concomitantly occurring with clear differences in glycaemic response between conditions, which is not adequately explored by the current data. For more frequent assessment of the glycaemic response the implementation of CGMs should be considered. This should produce a clearer and more accurate glycaemic response profile across the assessment period. A further advantage is that measurements could still be taken during a cognitive assessment, whereas the use of the fingerprick glucose method would require the interruption of cognitive testing.

Across the eighteen studies, a total of forty-nine cognitive tests were implemented with attention, executive function and episodic memory being the most investigated cognitive domains (see Table 2.3). Interestingly, the cognitive domain that returned the highest ratio of significant cognitive differences between GI/GL conditions relative to the frequency of assessments was immediate episodic memory. This finding is in line with previous research which demonstrates that the glucose facilitation effect is commonly observed in tests for verbal memory (Boyle et al., 2018; Hoyland et al., 2008; Messier, 2004; Riby, Meikle & Glover, 2004). A potential mechanism for the increased sensitivity of glucose administration in this cognitive domain could be the higher density of insulin receptors in the medial temporal regions of the brain, particularly the hippocampus, which is involved in episodic memory tasks (Craft & Watson, 2004; Dore et al., 1997; Marks et al., 1990; Messier, 2004). A rise in insulin levels that follows hyperglycaemia after meal consumption may result in increased glucose utilization by the hippocampus, which may lead to acute memory performance improvements (Craft et al., 1993). In the context of the reviewed research, differences in the insulin response induced by the GI/GL conditions could result in different levels of glucose utilization by the hippocampus via differing degrees of insulin receptor activation, especially given that there is a high density of insulin receptors in the medial temporal regions of the brain which are associated with episodic memory tasks (Boyle et al., 2018).

However, the large variation in both the cognitive tasks selected and the frequency of cognitive assessment across studies may also be a contributing factor to the mixed results reported. In terms of the selected cognitive tasks, some studies used well supported tests that have been previously tested for their cognitive performance detection sensitivity such as the Sternberg Paradigm (e.g. Cooper et al., 2015) whilst others included self-developed tasks which have not been substantiated by previous research (e.g. Brindal et al., 2012). The large variety of cognitive tasks used as well as the inclusion of customised/novel tasks makes overall interpretations of the reviewed research more difficult due to the expected variance in test sensitivity when assessing cognitive performance. Regarding task frequency, the number of cognitive performance assessments varied from a single examination (e.g. Micha et al., 2011) to a maximum of eight (Nilsson et al., 2012). Given that the focus of the reviewed research was comparing cognitive differences between GI/GL conditions, it is surprising that many studies only measured cognitive performance once or twice. The low frequency of cognitive testing across the morning results in a limited illustration of how cognitive performance alters throughout the postprandial phase. Furthermore, it is surprising that the assessment time points of cognitive performance and glycaemic response do not always occur at the same time across studies. For example, Micha et al. (2011) tested cognitive performance ninety minutes post meal consumption but took glycaemic measurements at 105- and 149- minutes post consumption. However, some studies did match glycaemic measurement with cognitive assessment times. For example, Lamport et al. (2013) took a glucose measurement at 30- and 120- minutes post food consumption, but also began cognitive assessment at the same time points. Measuring glucose concentrations immediately before cognitive assessments is advantageous for the researcher as it provides a clear image of the glycaemic response profile in each GI/GL conditions at these time points. As aforementioned, the use of a CGM could advance this procedure by allowing glycaemic measurements during the cognitive assessment

without the need to physically stop the participant and take a fingerprick capillary glucose sample.

To conclude, the current literature that investigates cognitive performance differences between glycaemic conditions generally favours the consumption of LGI/LGL meals, although some studies have reported beneficial cognitive effects following HGI/HGL meals, whilst some report no differences between glycaemic conditions. The large variation in results can be attributed to a number of methodological discrepancies across the reviewed research and include the failure of energy and macronutrient content matching between meals as well differences in glycaemic and cognitive assessments. Developmental changes in the brain (e.g. activity per unit weight) and the body (e.g. insulin sensitivity) often occurring in younger participants, as well as changes in glucose tolerance across the lifespan may also contribute to the mixed findings. A particular point to make is that there appears to be little emphasis on measuring the glycaemic response an appropriate number of times within the entire postprandial phase. This makes it difficult to compare any cognitive differences with particular parts of the postprandial glycaemic response, meaning that concomitant glycaemic differences could be occurring but are often missed by the current data. It could be speculated that the small frequency of glycaemic measurements reported here are due to the method of assessment i.e. a large number of capillary glucose finger prick samples may be difficult to conduct and painful for participants over a longer testing period. Therefore, future research should consider the use of flash or continuous glucose monitoring systems to allow multiple assessments to be taken with ease. This suggestion is supported by Brindal et al. (2012) who were able to take forty-one glycaemic measurements over a two-hundred minute period. Future research should also aim to describe dietary interventions in full detail to enable deeper comparisons and interpretations across studies. Given that the aim of a GI/GL implementation is to manipulate the glycaemic response, whether measured or inferred, it is also vital that future studies clearly state the GI/GL values associated with each experimental condition. Finally, the vast majority of the reviewed studies focus on the investigation of a single meal, particularly breakfast. There appears to be a distinct lack of multi-meal testing paradigms when investigating the relationship between glycaemic response, glucose tolerance and cognitive performance. Given that humans eat multiple meals throughout the day, it would be of interest to examine glycaemic response and cognitive performance across several meals. Therefore, it is critical that future research seeks to expand the largely used single meal paradigm to a multi-meal investigation in order to provide further knowledge of the relationship between glycaemic response, glucose tolerance and cognitive performance.

Table 2.2: A summary of eighteen studies examining glycaemic response, glucose tolerance and cognitive performance.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Benton et al. (2007)	19 total 9 male 10 female 6-7 years Mean 6.9yrs	Crossover	LGL: ham, cheese, bread, low-fat spread MGL: egg, bread, jam, low-fat spread, yoghurt HGL: cornflakes, milk, waffle, maple syrup	GI: not stated GL: LGL = 3 MGL = 12 HGL = 18	Not measured	Immediate Episodic Memory Delayed Episodic Memory Sustained Attention	Recall of Objects of the British Ability Scale Paradigm of Shakow	110-180	No significant cognitive differences between conditions.
Ingwersen et al. (2007)	64 total 26 male 38 female 6-11 years Mean 9.3yrs	Crossover	LGI: all bran cereal, milk HGI: coco pops cereal, milk	GI: LGI = 42 HGI = 77 GL: LGL = 7 HGL = 23	Not measured	Immediate Episodic Memory Delayed Episodic Memory Spatial Memory Working Memory Selective Attention	Word List Recall Spatial Working Memory task Numeric Working Memory task Word List Recognition Picture Recognition Digit Vigilance Simple & Choice reaction time	-30, 10, 70, 130	Lower accuracy of attention following HGI meal at 130 mins. Better memory performance following LGI meal at 10 and 130 mins.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Mahoney et al. (2005)	Expt 1: 30 total 15 male 15 female 9-11 years Mean not stated Expt 2: 30 total 15 male 15 female 6-8 years Mean not stated	Crossover	LGI: oatmeal cereal, milk HGI: ready-to-eat cereal, milk	Not stated	Not measured	Spatial Recall and Learning Immediate Recall, Attention and Working Memory Visual Spatial Perception Visual & Auditory Attention Episodic Memory	Self-developed spatial map task Digit Span Task Rey Complex Figure test Continuous Performance Task Self-developed prose memory test	All: 75 Visual Attention: 75, 95, 125	Expt 1: better short-term memory performance following LGI meal. Expt 2: better short-term memory and auditory attention following LGI meal.
Brindal et al. (2012)	39 total 26 male 13 female 10-12 years Mean 11.6yrs	Crossover	LGL: milk, yoghurt, cheese, bread, vegemite or jam, water MGL: yoghurt, cheese, bread, vegemite or jam, fruit drink, water HGL: white bread, margarine, vegemite or jam, fruit drink, water	GI: LGI = 48 MGI = 54 HGI = 67 GL: LGL = 18 MGL = 24 HGL = 33	0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200	Speed of processing Working memory Perceptual speed Attention switching	Simple & Choice Reaction Time Attention switching task Immediate word recall Digit Span Task Visual	0, 60, 120, 180	No significant cognitive differences between conditions.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Cooper at al. (2015)	42 total 20 male 22 female 11-13 years Mean 12.4yrs	Mixed	LGI: muesli, apple, 1% fat milk HGI: cornflakes, white bread, margarine, 1% fat milk Matched for energy, protein, and fat. CHO was provided as 1.5g/kg body mass	GI: LGI = 48 HGI = 72 GL: LGL = 36 HGL = 54	0, 30, 60, 120	Visual Attention Alternating Attention, Selective Attention and Impulsivity Working Memory and Speed of information processing	Visual search task Stroop task Sternberg paradigm	30, 120	Better performance on complex stroop task levels after LGI meal and exercise. Better Sternberg paradigm performance following LGI meal.
Micha et al. (2010)	60 total 24 male 36 female 11-14 years Mean 13yrs	Between Groups	Categorized breakfast and snack (if consumed) eaten on the day of testing into 4 groups: 1. LGI + LGL 2. LGI + HGL 3. HGI + LGL 4. HGI + HGL	GI: LGI < 61 HGI > 51 GL: GL < 27 HGL > 27	105, 149	Verbal Fluency Verbal Memory Attention and Impulsivity Visual Reasoning and Nonverbal Intelligence Speed of Processing, Visual Attention	Word generation task Immediate & Delayed word recall Stroop task Matrices Speed of processing task Serial sevens	90	Better immediate word recall following HGI meals. Better matrices performance following HGL meals. Better speed of information processing and serial sevens performance following LGI and HGL meals.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Cooper et al. (2012)	41 total 18 male 23 female 12-14 years Mean 12.8yrs	Crossover	LGI: muesli, milk, apple HGI: cornflakes, white bread, margarine, milk Matched for energy, protein, and fat. CHO was provided as 1.5g/kg body mass	GI: LGI = 48 HGI = 72 GL: LGL = 36 HGL = 54	Not measured	Alternating Attention, Selective Attention and Impulsivity Working Memory and Speed of information processing Attention and Response Time	Stroop task Sternberg paradigm Flanker task	30, 120	Improved stroop task accuracy and response time following LGI meal. Accuracy was maintained better and a greater improvement in response time was seen in the sternberg paradigm following LGI meal.
Micha et al. (2011)	74 total 37 male 37 female 11-14 years Mean 12.6yrs	Crossover	Given different amounts of muesli, cornflakes, milk, apple juice and sugar dependent on group: 1. LGI + LGL 2. LGI + HGL 3. HGI + LGL 4. HGI + HGL	GI: LGI = 48 HGI = 61 GL: 1 = 21 2 = 41 3 = 28 4 = 55	0, 90	Verbal Fluency Immediate & Delayed Verbal Recall Sustained & Selective Attention Visual Reasoning & Intelligence Processing Speed	Word Generation Task Immediate & Delayed Word Recall Stroop Task Matrices Speed of Information Processing Serial Sevens	90	Better word generation performance following LGI meals. Better stroop task performance following HGI meals. Better speed of processing and serial sevens performance after HGI meals.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Smith & Foster (2008)	38 total 19 male 19 female 14-17 years Mean 15.6yrs	Between Groups	LGI: 30g Keloggs All Bran, Milk HGI: 30g Corn Flakes, Milk	GI: LGI = 30 HGI = 77 GL: Not stated	-10, 10, 50, 90	Verbal Recall	The California Verbal Learning Task	20, 60, 100	A significantly higher proportion of words were recalled at 100 minutes following HGI meal consumption.
Lamport et al. (2011)	14 total 14 male 0 female 19-28 years Mean 22.4yrs	Crossover	Evening meal: LGI: Pasta, Turkey, Cheese, Lettuce, Pasta Sauce, Pear, Apple Juice HGI: White Bread, Turkey, Cheese, Lettuce, Banana, Lucozade Following standard HGI breakfast: Corn Flakes, Skimmed Milk, White Bread, Flora, Jam, Lucozade	Evening: GI: LGI = 47 HGI = 72 GL: LGL = 63 HGL = 96 Breakfast: GI = 75 GL = 106	Evening meal: 30, 45, 60, 75, 90 minutes Breakfast: -15, 0, 15, 30, 45, 60, 75 minutes	Verbal Memory Alternating Attention Visual Recognition Memory	Word Recognition Attention Switching task Visual Verbal Learning task	Evening: Not carried out Breakfast: -15, 30 minutes	The HGI evening meal showed a non-significant association with better verbal recall the following morning. A non-significant tread was found suggesting slightly more words were remembered in the HGI condition, but only after breakfast consumption.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Lamport et al. (2014a)	65 total 0 male 65 female 30-50 years Mean not stated	Crossover	LGL: toasted soya and linseed bread, strawberry yoghurt HGL: lucozade energy original drink Water breakfast: water	GI: LGI = 32 HGI = 95 GL: LGL = 12 HGL = 71	0, 15, 30, 60, 90, 120, 150, 180	Immediate & Delayed Verbal Memory Spatial memory Psychomotor function Executive function	Visual Verbal Learning test Visual Spatial Learning test Corsi Block Tapping test Tower of Hanoi Grooved Pegboard Psychomotor test Word Recognition	30, 120	Verbal memory: LGL meal attenuated deficits seen at 120 minutes in combined impaired glucose tolerance (IGT) and high waist circumference group. Spatial memory: The IGT/HWC group displayed impairments on the VSLT at 120 minutes following the HGL meal.
Lamport et al. (2014b)	65 total 18 IGT 47 controls 0 male 65 female 30-50 years No Mean	Crossover	LGL: toasted soya and linseed bread, strawberry yoghurt HGL: lucozade Water breakfast	GI: LGI = 32 HGI = 95 GL: LGL = 12 HGL = 71	0, 15, 30, 60, 90, 120, 150, 180	Immediate & Delayed Verbal Memory	Visual Verbal Learning task Source Monitoring task	30, 120	No significant cognitive differences between conditions.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Lamport et al. (2013)	34 total 24 T2DM 10 controls 16 male 18 female 45-77 years Mean not stated	Crossover	LGL: toast, strawberry yoghurt HGL: lucozade energy original drink Placebo: Water	GI: LGI = 32 HGI = 95 GL: LGL = 12 HGL = 71	0, 15, 30, 60, 90, 120, 150, 180	Immediate & Delayed Verbal memory Spatial memory Psychomotor skill Executive function	Visual Spatial Learning test Visual Verbal Learning test Corsi Block Tapping test Tower of Hanoi Grooved Pegboard Psychomotor test Source Monitoring Paragraph Recall	30, 120	Delayed verbal memory: T2D group recalled significantly less words than NGT group at 120 minutes following the water meal. Psychomotor skill: Significantly faster completion times following LGL meal compared to water meal. No significant differences between GL conditions.
Young & Benton (2014)	155 total 59 male 96 female 45-80 years	Between Groups	All: toast, jam, yoghurt, orange flavoured drink 15g added: LGL: isomaltose MGL: sugar HGL: glucose	GI: Not stated GL: LGL = 27.2 MGL = 41.3 HGL = 54.4	0, 30, 60, 90, 120, 150, 180	Episodic memory Working memory Reaction time Sustained attention	Immediate & Delayed recall Serial Sevens Simple & Choice Reaction Time Information processing task	30, 105, 195	Episodic Memory: Good GT, BG above baseline group: better memory at 30, 105 and 195 minutes after LGL. Better GT, BG below baseline group: poorer memory at 195 after HGL meal.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Papanikolaou et al. (2006)	21 T2DM 10 male 11 female No range Mean 65yrs	Crossover	LGI: Pasta HGI: White Bread Placebo: Water	GI: LGI = 43 HGI = 73 GL: LGL = 22 HGL = 37	-5, 15, 62, 100, 138	Verbal Memory Working Memory Motor Function Sustained and Selective Attention	Hopkins Verbal Learning Task Wechsler Memory Scale Verbal Paired Associates Digit Span Trail Making Task Test of Everyday Attention	HVLT, WMS and VPA: 15, 62, 100 DS, TMT and TEA: 62 100	HVLT: Better Immediate Verbal Recall in the LGI condition at first three recalls. Better Delayed Verbal Recall after second delay (100 minutes). WMS: Better Delayed Memory in the LGI condition after first delay (62 minutes) and second delay (100 minutes). DS: Better Working Memory in the LGI condition after first delay (62 minutes). TMT: A greater improvement was observed between 2 administrations of part B for the LGI condition.
Nilsson et al. (2012)	40 total 12 male 28 female 49-71 years No Mean	Crossover	LGI: White Bread enriched with guar gum HGI: White Bread	GI: LGI = 45 HGI = 100 GL: Not stated	0, 15, 30, 45, 60, 90, 120, 150	Working Memory Selective Attention	Reading Comprehension task Custom Picture task	WM: 90, 135, 180, 225 SA: 75, 120, 165, 210	Significantly better performance on the custom picture task at 120 minutes following a LGI meal.

Table 2.2: Continued.

Source	Sample	Design	CHO Intervention	GI/GL values	Glucose Sampling	Cognitive Domain	Cognitive Test	Test Time (mins post food)	Findings
Kaplan et al. (2000)	20 total 10 male 10 female 60-82 years Mean 72.3yrs	Crossover	50g portion: LGI: Barley HGI (1): Instant Mashed Potato HGI (2): Glucose drink Placebo: Water	GI: LGI = 25 HGI1 = 83 HGI2 = 100 GL: Not stated	0, 15, 60, 105	Verbal Recall Attention, Mental Flexibility, Visual Spatial Skills Sustained Attention	Custom Word Recall Wechsler Memory Scale Trail Making test Custom Attention task	15, 60, 105	No significant cognitive differences between conditions.
Benton et al. (2003)	106 total 0 male 106 female No range Mean 21.1yrs	Between Groups	Two breakfasts differing in type of CHO (SAG vs. RAG)	GI: LGI = 42 HGI = 66 GL: Not stated	0, 20, 50, 80, 140, 200, 230	Immediate & Delayed Verbal Memory	Word List Recall	30, 90, 150, 210	Better recall of concrete words at 210 minutes following LGI meal. Better recall of abstract words at 30, 90, 150 and 210 minutes following LGI meal.

*CHO = Carbohydrate, GI = Glycaemic Index, GL = Glycaemic Load

Table 2.3: The number of significant differences between GI/GL conditions for each cognitive domain and each cognitive test in studies examining glycaemic response, glucose tolerance cognitive performance.

Cognitive Domain	Cognitive Task	Significant Findings/Frequency Implemented
Immediate Episodic Memory (17/41)	Recall of Objects (BAS)	0/1
	Word List Recall	9/17
	Custom Prose Recall	6/6
	Visual Verbal Learning Task	1/6
	Paragraph Recall	0/2
	Hopkins Verbal Learning Task	1/1
	Wechsler Memory Scale	0/4
	Verbal Paired Associates	0/1
	Custom Word Recall	0/3
Delayed Episodic Memory (12/42)	Recall of Objects (BAS)	0/2
	Word List Recall	6/13
	Word List Recognition	1/8
	Picture Recognition	1/4
	California Verbal Learning Test	1/3
	Source Monitoring Task	0/4
	Paragraph Recall	0/2
	Hopkins Verbal Learning Task	1/2
	Wechsler Memory Scale	2/2
	Verbal Paired Associates	0/2
Verbal Fluency (0/2)	Word Generation	0/2
Visuo-spatial Memory (1/18)	Spatial Working Memory	0/4
	Custom Spatial Map	0/6
	Visual Spatial Learning Test	1/4
	Corsi Block Tapping Test	0/4
Executive Function (5/39) (includes working memory)	Numeric Working Memory	0/4
	Digit Span	1/12
	Rey Complex Figure	0/6
	Sternberg Paradigm	2/4
	Serial Sevens	2/5
	Tower of Hanoi	0/4
	Reading Comprehension	0/4
Attention and Vigilance (12/53)	Paradigm of Shakow	0/1
	Digit Vigilance	1/4
	Continuous Visual Performance	0/6
	Continuous Auditory	3/6
	Performance	0/6
	Attention Switching	1/8
	Custom Visual Inspection	0/2
	Visual Search	4/6
	Stroop Task	1/2
	Matrices	2/5
	Information Processing	0/2
	Flanker Task	0/2

	Test of Everyday Attention	0/3
	Custom Attention Task	
Psychomotor Skill (1/11)	Grooved Pegboard	0/4
	Psychomotor Test	0/2
	Trail Making Task	1/5
Reaction Time (2/22)	Simple Reaction Time	1/11
	Choice Reaction Time	1/11

*Tests were categorised into each domain based on the authors' categorisations, and categorisations by Lezak et al. (2004) and Boyle at al. (2018). BAS = British Ability Scale

2.3 Glycaemic response, glucose tolerance and mood

In addition to assessing cognitive performance, the research conducted for this thesis also measures subjective mood state. When conducting cognitive research, it is important to also implement subjective measures of cognitive function because a standardised cognitive task battery usually only monitors outcomes of objective cognitive performance e.g. accuracy scores or reaction time. By focusing solely on objective cognitive measures through the use of a standardised cognitive task battery, some significant cognitive differences between conditions may not be detected. For example, a participant may produce similar cognitive performance on an attention task between a LGI and HGI condition but also feel significantly less alert and content in the HGI condition. In this scenario, if subjective alertness and contentment were not measured, then the researcher would not be able to interpret that the LGI condition was indeed more beneficial to cognition compared to the HGI condition. It could even be argued that subjective measures such as alertness are of greater interest than standardised cognitive task performance in some instances. For example, two diets that are associated with better performance in sustained attention would be of interest to an elite endurance athlete. However, the athlete would be more likely consume one of the diets if it was also associated with higher alertness and lower fatigue levels. Therefore, the research conducted for this thesis (Chapter 4-7) also investigated the relationship between glycaemic response and subjective mood by implementing Bond & Lader visual analogue scales (VAS) (Bond & Lader, 1974) (see Chapter 3 for description).

Whilst the association between a poor glucose tolerance status such as T2DM and increased rates of clinical mood disorders is well established (Maraldi et al., 2007), it is important to note that the focus of this thesis is the relationship between acute glycaemia and subjective mood. Thus, thirteen acute studies are summarised here (see Table 2.4). Out of the thirteen studies, the majority implemented a healthy sample (Cooper et al., 2011, 2015; Defeyter & Russo, 2013; Micha et al., 2010, 2011; Nabb & Benton, 2006; Owen et al., 2013; Smith et al., 1994; Sunram-Lea et al., 2011; Young & Benton, 2014). The findings from the studies in healthy samples can be summarised two-fold; (i) the consumption of food, and thus the presence of a glycaemic response, appears to be associated with improved mood compared to meal omission conditions whilst (ii) the comparison of mood between GI/GL conditions has returned mixed results with studies either finding no significant differences or improved mood in either a LGI/LGL or HGI/HGL condition. However, it is important to consider that very few studies involved the comparison between a LGI/LGL condition with a HGI/HGL condition, thus

knowledge of this area is extremely limited. This is surprising given that longitudinal research has reported that a higher dietary GI/GL is associated with increasing rates of negative affect over a testing period of years (Cheatham et al., 2009; Gangwisch et al., 2015). It should also be noted that the studies that did compare acute GI/GL conditions all implemented a single meal testing paradigm, leaving the relationship between glycaemia and subjective mood largely unexplored over the course of several consecutive meals.

Out of the thirteen reviewed studies, only three explored glycaemic response and subjective mood in those with T2DM in an acute setting (Greenwood et al., 2003; Pais et al., 2007; Sommerfield et al., 2004). Although not reviewed here, it should be noted that the large majority of research involving T2DM samples is longitudinal or cross-sectional in nature with these studies focusing on the relationship between long-term glycaemic control and the prevalence of clinical mood disorders rather than measuring acute glycaemic response and subjective mood state. These studies have consistently reported a positive association between poorer glycaemic control and clinical mood disorder rates in those with T2DM (Balhara & Sagar, 2011; Connell et al., 1990; Fisher et al., 2007, 2010; Gonzalez et al., 2015; Gross et al., 2005; Katon et al., 2004; Miyaoka et al., 1997; Papelbaum et al., 2011; Trento et al., 2012; Van der Does et al., 1996; Zhang et al., 2015). However, there is a distinct lack of studies that implement the GI/GL concept to investigate acute glycaemic response and subjective mood in T2DM.Instead, two of the three studies reviewed here used glycaemic clamp techniques to maintain acute euglycaemic and hyperglycaemic conditions (Pais et al., 2007; Sommerfield et al., 2004). The findings from these studies appear conflicting with Pais et al. (2007) reporting better mood during the hyperglycaemic condition whilst Sommerfield et al. (2004) found the hyperglycaemic condition to be associated with worsened mood. Although, it should be noted that higher glucose concentrations were maintained during the hyperglycaemic condition in the study conducted by Sommerfield et al. (2004) i.e. 16.5mmol/L rather than 10.5mmol/L. Therefore, it could be reasonably inferred from the findings that a higher glycaemic response may be associated with more negative mood outcomes than a lower glycaemic response. Whilst the findings generally support a relationship between higher glucose concentration levels and increased negative mood outcomes in those with T2DM, the studies do not provide information on a potential relationship between an acute glycaemic response and mood. Furthermore, the only study that provided meals to a T2DM sample found no significant mood differences between a meal consumption and omission condition but did not monitor the postprandial glycaemic response (Greenwood et al., 2003). Further research into this area is required in order to establish whether acute differences in glycaemic response between glycaemic conditions are associated with significant differences in subjective mood for those with T2DM.

The clearer association between mood and longer term glycaemic measures (e.g. HbA1c levels) suggest that the potential underlying mechanisms moderating the relationship between glycaemic response, glucose tolerance and mood require repeated injury to produce significant mood differences. For example, a subtle increase in the glucocorticoid cortisol that can occur when consuming a HGI/HGL meal compared to a LGI/LGL meal (Micha et al., 2011) may not be enough to significantly affect mood. However, chronic dysfunction of the hypothalamus-pituitary- adrenal (HPA) axis would result in a long-term increase of circulating cortisol levels, which has been associated with prevalent negative mood outcomes

(Ellenbogen et al., 2004, 2010; Handley et al., 1980; Reagan et al., 2008; Van Eck et al., 1996). Given that the majority of reviewed studies examined an ostensibly healthy sample with normal glucose tolerance, this would explain the lack of significant mood differences that are reported in acute settings. This is further supported by Nabb & Benton (2006) who reported that participants who were classified as good glucose regulators within the normal glucose tolerance range displayed better mood than those identified as poor glucose regulators. Overall, it is plausible that differences in glucose tolerance, even within a normal range, share a clearer relationship with mood outcome measures than acute differences in glycaemic response due to the underlying mechanisms being more chronic in nature (see Section 2.4 for details on mechanisms).

It is likely that underlying mechanisms require repeated injury over an extended period of time to result in significant changes in mood (see Section 2.4), although it is also plausible that the methodology of the acute studies plays a role in the lack of significant findings. For example, not all studies matched calorie and macronutrient contents between glycaemic conditions. Nabb & Benton (2006) provided participants with one of eight meals that varied in CHO content and reported that higher amounts of CHO were associated with increased fatigue. Furthermore, the studies that compared meal consumption and omission conditions consistently reported that more positive affect was observed in the meal consumption condition (Cooper et al., 2011; Defeyter & Russo, 2013; Smith et al., 1994). Taken together, these findings suggest that the presence of a glycaemic response is associated with better mood than the absence of one but the glycaemic response profile, affected by varied energy and macronutrient content, is also important. Previous research has also indicated that both fat and protein share a negative association with postprandial glucose rise by augmenting the insulin response (Gunnarsson et al., 2006; Jenkins et al., 1981). An augmented insulin response can lead to an insulin spike, where there is a large concentration of circulating insulin that can often lead to a period of reactive hypoglycaemia (Crofts, 2015; Cryer, Fisher & Shamoon, 1994). Acute hypoglycaemia has been previously associated with a significant increase in subjective tension and decreases in energetic arousal and hedonic tone (Gold et al., 1995). Therefore, future research that investigates the relationship between the acute glycaemic response and mood needs to ensure that test meals are matched in their energy and macronutrient content. This would allow researchers to then implement a concept such as GI/GL to manipulate the glycaemic response profile and measure concurrent mood whilst avoiding confounding effects of energy/macronutrient variation on the glycaemic response and mood outcome measures.

The selection of mood assessment tools also needs careful consideration, with the reviewed studies using previously validated and reputable questionnaires/inventories. For example, three studies chose the Bond and Lader VAS to assess mood (Bond & Lader, 1974). The Bond and Lader VAS has previously been shown to have good internal consistency with a Cronbach's $\alpha = 0.77\text{-}0.93$ (Miroddi et al., 2014), although test-retest reliability is not feasible as the assessment tool is focused on a "here and now" rating of subjective mood (Bond & Lader, 1974). The focus on the "here and now" state of this assessment tool is advantageous as it is not measuring a stable phenomenon or trait, potentially making it a useful questionnaire/inventory when making multiple assessments during glycaemic conditions of an acute study. This would allow mood outcomes to be compared across different phases of the

glycaemic response if the VAS is completed subjectively and honestly multiple times throughout postprandial glycaemia. However, as is the case with all self-report mood questionnaires/inventories the method of administration can affect the subsequent data obtained. Bowling (2005) highlights that self-reported scores can be exaggerated or minimized if a questionnaire is completed in front of other people due to social beliefs or expectations, leading to different responses compared to administration via post or internet. It has also been suggested that physical symptoms such as fatigue can affect the self-reporting of mood, which could lead to the misinterpreting of such symptoms as negative mood outcomes (Moore, Moore & Shaw, 1998). Therefore, it is wise for future research to also measure subjective outcomes such as fatigue or sleepiness when investigating glycaemic response and mood to allow greater confidence in interpreting any significant differences in mood between conditions, without the potentially confounding effect of unnoticed differences in symptoms such as fatigue or sleepiness.

To conclude, the current literature investigating the relationship between acute glycaemia and subjective mood is limited. In healthy individuals, it appears that meal consumption is beneficial for subjective mood outcomes compared to meal omission conditions. Whereas, in T2DM samples there is an association between higher glucose concentrations and increased negative affect. There is currently a finite number of studies which utilise the GI/GL concept to investigate the relationship between postprandial glycaemia and subjective mood, while the few studies that have taken this approach are confined to the examination of a single meal. The current findings are mixed with some studies reporting improved mood following either a LGI/LGL or HGI/HGL condition, whilst others have found no significant differences between glycaemic conditions on any subjective mood measure. Thus, the present thesis utilises the GI/GL concept and extends the single meal testing paradigm to investigate the relationship between glycaemic response and subjective mood during two glycaemic conditions, each consisting of three consecutive meals. The selection and implementation of subjective mood assessment tools was carefully considered and subjective outcomes such as sleepiness were also measured (see Chapter 3).

Table 2.4 A summary of thirteen studies investigating the relationship between glycaemic response, glucose tolerance and mood.

Source	Sample	Design	Intervention or Procedure	Glucose Sampling	Mood Measures	Mood Assessment Time	Findings
Pais et al. (2007)	15 T2DM 7 male 8 female 39-69 years Mean 56yrs	CO	Mood assessed during two conditions: Euglycaemia (5mmol/L) Hyperglycaemia (10.5mmol/L)	Each glycaemic condition was maintained for the duration of cognitive assessment.	123AC SQSQ	One measurement per glycaemic condition.	Significantly higher well-being and a trend for less anger during hyperglycaemia.
Sommerfield et al. (2004)	20 T2DM 12 male 8 female 53-72 years Mean 61.5yrs	CO	Mood assessed during two conditions: Euglycaemia (4.5mmol/L) Hyperglycaemia (16.5mmol/L)	Each glycaemic condition was maintained for the duration of cognitive assessment.	UWIST	One measurement per glycaemic condition.	Participants displayed significantly lower happiness and alertness during hyperglycaemia. Significantly higher levels of agitation were reported during hyperglycaemia.
Micha et al. (2011)	74 total 37 male 37 female 11-14 years Mean 12.6yrs	CO	Muesli, cornflakes, milk, apple juice and sugar groups: 1. LGI + LGL 2. LGI + HGL 3. HGI + LGL 4. HGI + HGL	0 and 90 minutes post meal.	Questionnaire developed from: POMS-BI ADAC	90 minutes post meal.	Participants felt significantly less nervous, more alert and happier following LGI meals. Participants reported feeling more confident and less sluggish after HGL meals.

Table 2.4: Continued.

Source	Sample	Design	Intervention or Procedure	Glucose Sampling	Mood Measures	Mood Assessment Time	Findings
Sunram-Lea et al. (2011)	30 total 6 male 24 female 18-25 years Mean 20yrs	CO	Five different doses of glucose via drink: 0g, 15g, 25g, 50g, and 60g	0, 22, 32 and 47 minutes post drink.	BLVAS	0 and 120 minutes post drink.	No significant mood differences between any of the five conditions.
Nabb & Benton (2006)	168 total 0 male 168 female No range Mean 20.4yrs	BG	Participants consumed one of eight meals with varied CHO and fibre content. CHO doses: 10g, 30g, 50g Fibre doses: 1.5g, 6g, 13g	0, 20, 50, 80 and 110 minutes post meal.	POMS	0, 20, 50, 80 and 110 minutes post meal.	Those with better glucose tolerance within the sample reported better mood. Higher amounts of CHO were associated with higher levels of tiredness.
Cooper et al. (2015)	42 total 20 male 22 female 11-13 years Mean 12.4yrs	M	Participants allocated to a LGI or HGI meal condition and performed both exercise and resting trials.	0, 30, 60, 120 minutes post meal.	ADAC	0, 30, 60 and 120 minutes post meal.	Significantly higher levels of energy and lower levels of tiredness, tension and calmness 30 minutes post consumption in both glycaemic conditions, regardless of exercise.

Table 2.4: Continued.

Source	Sample	Design	Intervention or Procedure	Glucose Sampling	Mood Measures	Mood Assessment Time	Findings
Cooper et al. (2011)	96 total 36 male 60 female 12-15 years Mean not stated	CO	Two glycaemic conditions: No breakfast Ad-libitum consumption of cereal, toast, yoghurt and fruit juice.	0 and 120 minutes post meal.	ADAC	0 and 120 minutes post meal.	Significantly higher levels of energy and lower levels of tiredness reported in the breakfast condition compared with no breakfast.
Micha et al. (2010)	60 total 24 male 36 female 11-14 years Mean 13yrs	BG	Categorized breakfast and snack eaten on the day of testing into 4 groups: 1. LGI + LGL 2. LGI + HGL 3. HGI + LGL 4. HGI + HGL	105 and 149 minutes post meal.	Questionnaire developed from: POMS-BI ADAC	90 minutes post meal.	No significant mood differences between glycaemic conditions were observed.
Young & Benton (2014)	155 total 59 male 96 female 45-80 years Mean not stated	BG	Toast, jam, yoghurt, orange drink with 15g: LGL: isomaltose MGL: sugar HGL: glucose	0, 30, 60, 90, 120, 150 and 180 minutes post meal.	POMS	30, 105 and 195 minutes post meal.	Those who consumed isomaltose and sucrose were more agreeable than those who consumed glucose.

Table 2.4: Continued.

Source	Sample	Design	Intervention or Procedure	Glucose Sampling	Mood Measures	Mood Assessment Time	Findings
Owen et al. (2013)	24 total Genders not stated 18-30 years Mean 20yrs	CO	Three different doses of glucose via drink: 0g, 25g and 60g	0, 15 minutes post drink. Third reading upon end of cognitive assessment.	BLVAS	15 minutes post drink.	No significant differences between glycaemic conditions.
Defeyter & Russo (2013)	40 total 19 male 21 female 13.2-15.6 years Mean 14.2yrs	CO	Two meal conditions: Meal omission LGI breakfast	Not measured.	BLVAS	-30 and 135 minutes post meal.	Significantly higher levels of alertness and contentment were reported in the LGI condition.
Smith et al. (1994)	48 total 24 male 24 female No range Mean not stated	BG	Participants consumed one of six meals: No breakfast, cooked breakfast or cereal/toast. With or without caffeine.	Not measured.	18BLR	-30, 60 and 120 minutes post meal.	Participants in the cooked breakfast condition felt significantly more contented, interested, sociable and outward-going than the no breakfast and cereal/toast conditions.

Table 2.4: Continued.

Source	Sample	Design	Intervention or Procedure	Glucose Sampling	Mood Measures	Mood Assessment Time	Findings
Greenwood et al. (2003)	19 T2DM 12 male 7 female No range Mean 63yrs	CO	Two meal conditions: Meal omission Bagel and grape juice breakfast	Fasting glucose measured HbA1c measured	GVAVAS	30 minutes post meal.	No significant differences between glycaemic conditions.

* Research Designs: BG = Between Groups, CO = Cross-over, M = Mixed. Mood Measures: ADAC = Activation-Deactivation Adjective Checklist, BLVAS = Bond & Lader Visual Analogue Scales, B8IS = Burnam 8 Item Scale, CES-D = Center for Epidemiologic Studies Depression tool, GVAVAS = Global Vigor and Affect Visual Analogue Scale, POMS = Profile of Mood States, POMS-BI = Profile of Mood States Bipolar form, SQSQ = Semi-Quantitative Symptom, Questionnaire, UWIST = University of Wales Institute of Science and Technology mood checklist 18BLR = 18 Bipolar Line Ratings, 123AC = 123 Adjectives Checklist.

2.4 Mechanisms associating glycaemic response, glucose tolerance, cognitive performance and mood

There are likely multiple contributing underlying mechanisms that associate glycaemic response, glucose tolerance, cognitive performance and mood. These relate to glucose transport across the blood brain barrier (BBB), neuroinflammation, the synthesis and regulation of neurotransmitters, dysregulation of the hypothalamic pituitary-adrenal (HPA) axis, variations in insulin sensitivity and the adverse effects of chronic hyperglycaemia. These mechanisms and their potential effects on cognitive performance and mood are detailed below.

2.4.1 Glucose transport across the blood brain barrier (BBB)

The brain's main metabolic fuel is glucose (Amiel, 1994; Gomez-Pinilla, 2008; Maclean et al., 2003; Seiber & Traystman, 1992). Due to the limited glycogen stores that the brain possesses, it largely relies on exogenous glucose transported from the blood across the BBB (Weiss, 1986). The relationship between peripheral and neuronal glucose levels has been shown to be linear in humans (Seaquist et al., 2001). Furthermore, animal studies have identified a steady ratio between neural and peripheral blood glucose, with brain extracellular glucose concentrations being 20-30% of peripheral blood glucose levels in rodents (Abi-Saab et al., 2002; Gruetter et al., 1996; Harada et al., 1993; Jacob et al., 2002; Messier, 2004). This suggests that increasing glucose levels in the blood, by consuming food, could lead to increases in brain extracellular glucose concentrations, and subsequent improvements in cognitive performance. However, previous research has indicated that neural activity is the important determinant of neuronal glucose uptake in normoglycaemic conditions rather than extracellular brain glucose concentrations (Messier, 2004). Indeed, previous reviews of the literature have continuously highlighted the importance of cognitive load as a moderator in the relationship between glycaemic response, glucose tolerance and cognitive performance (Boyle et al., 2018; Sunram-Lea & Owen, 2017). An increase in cognitive load has been associated with reduced glucose concentrations in neural areas such as the visual cortex during visual stimulation (Chen et al., 1993) and hippocampus during memory activation (McNay et al., 2000). This supports neural activity as the determinant in glucose uptake rates in the brain, which are increased during periods of higher cognitive effort. Given the link between neural effort and glucose uptake rates, it is vital that the brain is supplied with enough glucose from the blood across the BBB. Glucose is transported across the BBB through GLUT 1 transporters, which are highly expressed in its' endothelial cells (Giaume et al., 1997; Serlin et al., 2015; Shah, DeSilva & Abbruscato, 2012; Virgintino et al., 1997). When the brain demands more glucose, the number of GLUT 1 transporters in contact with the blood rises to increase glucose transport rates across the BBB (Patching, 2017; Simpson et al., 1999).

Previous research has shown that the rate at which glucose crosses the BBB is reduced in those with abnormal glucose tolerance, such as T2DM (Convit, 2005). The current evidence indicates that this may be the result of BBB endothelium dysfunction (Baron, 1996; Benatti et al., 2016; Brownlee, 2001; Cohen, 1993; Huber et al., 2008; Su et al., 2008) and a subsequent

reduction in GLUT 1 transporter availability and function (Duelli et al., 2000; Hwang et al., 2017; McCall, 1992; Mooradian & Morin, 1991; Prasad et al., 2014; Shah, DeSilva & Abbruscato, 2012). Although it is currently unclear why poorer glucose tolerance may lead to BBB dysfunction, Brownlee (2001) suggests that chronic hyperglycaemia is toxic to the BBB endothelial cells via an increased production of harmful super-oxides. Given the link between neural effort and neuronal glucose uptake, such dysfunctional mechanisms could lead to an inadequate supply of glucose to the brain during periods of high cognitive demand. This would explain the previously highlighted relationship between glucose tolerance abnormalities and cognitive deficits (Boyle et al., 2018; Convit, 2005; Lamport et al., 2009). Interestingly, older age has also been linked with alterations in BBB endothelium permeability (Farrall & Wardlaw, 2009). Thus, this mechanism may explain the increased cognitive impairments reported in older individuals with poorer glucose tolerance (Awad et al., 2004; Biessels et al., 2008; Boyle et al., 2018; Lamport et al., 2009; Sunram-Lea & Owen, 2017; Wrighten et al., 2009).

This mechanism may also explain why glucose consumption is often reported as most beneficial to those with poorer glucose tolerance (Awad et al., 2002; Lamport et al., 2009). Given the linear relationship between peripheral and neural glucose concentrations, it is plausible that the consumption of glucose before or during cognitive effort would speed up the re-establishment of the ratio between the two. The first step of this process would be increases in peripheral glucose concentrations following the consumption and digestion of glucose. This would result in an immediate increase in the difference between peripheral and neural glucose concentrations. At this point, it would be expected that more glucose transporters would translocate to the blood side of the BBB in an effort to re-establish this ratio (Patching, 2017, Pelligrino et al., 1992). Theoretically, this would lead to a faster restoration of depleted neural glucose levels, which occurred as a result of the cognitive demand. Therefore, those with BBB dysfunction would stand to benefit more from glucose consumption than healthy individuals due to their reduced ability to increase depleted neural glucose concentrations (Lamport et al., 2009). Indeed, animal research has shown that reductions in hippocampal extracellular glucose levels (12% young rats, 48% old rats), seen during a maze task, can be abolished through peripheral injections of glucose (McNay et al., 2000, 2001). The same researchers also reported improved memory performance on the maze task for both young and old rats following glucose injection.

To summarise, the current evidence suggests that poorer glucose tolerance (e.g. T2DM) may lead to BBB injury. This can be characterised as dysfunction of the BBB endothelium, which reduces GLUT 1 transporter availability. The result is a reduction in the transport of glucose from the peripheral blood into the brain, leading to an inability to restore extracellular glucose at an sufficient rate. As neural effort is an important determinant of neuronal glucose uptake, cognitive deficits can become more apparent during a higher cognitive load. Taken together, this offers an explanation as to why those with poorer glucose tolerance appear to benefit more from glucose consumption than healthy individuals during cognitive testing (Lamport et al., 2009). Previous research has also indicated that older age, obesity, hypertension and dyslipidaemia can adversely affect endothelial function (Awad et al., 2004; Biessels et al., 2008; Boyle et al., 2018; Lamport et al., 2009; Panza et al., 1993; Sunram-Lea & Owen, 2017; Versari et al., 2009; Wrighten et al., 2009) which suggests an interaction between these factors and poor glucose tolerance could lead to further cognitive impairment.

2.4.2 Neuroinflammation

As aforementioned in Section 2.4.1.1, poorer glucose tolerance is associated with BBB endothelium dysfunction (Baron, 1996; Brownlee, 2001; Cohen, 1993; Huber et al., 2008; Su et al., 2008). This dysfunction can lead to increased permeability of the BBB, with a recent research proposing that this increases neuroinflammatory burden (Benatti et al., 2016 De Felice & Ferreira, 2014). An increase in BBB permeability has been observed in many systemic disorders such as T2DM (Rosenberg, 2012), and it is considered that altered BBB vascular homeostasis is the result of increased oxidative stress (i.e. overproduction of reactive oxygen species; ROS) during repeated and extended hyperglycaemia (Kadoglou et al., 2005; Ristow, 2004). The increased BBB permeability then allows plasma components, immune molecules and cells to enter the brain (Abbot et al., 2010; da Fonseca et al., 2014). These components can then activate resident microglia, which are non-neuronal cells involved in the immune defence of the brain (Abbott & Friedman, 2012; Delpech et al., 2015; Skaper, Facci & Giusti, 2014). Microglia are particularly responsive to inflammatory signals and can be primed to respond more severely with each subsequent disruption of the brain environment (Skaper, Facci & Giusti, 2014). Research has found an overproduction of the main pro-inflammatory cytokines produced by primed microglia; interleukin (IL- 1β), IL-6, tumor necrosis factor-α (TNF-α) in brains of patients suffering with depression (Zunszain et al., 2011). Human studies have reported elevated levels of TNF-α in the blood of patients with T2DM (Chen et al., 2007), and a positive correlation between IL- 1β levels and the pathogenesis of T2DM (Boni-Schnetzler et al., 2008; Hivert et al., 2009). Taken together, these results suggest that frequent and extended hyperglycaemia, often occurring in T2DM, can increase the neuroinflammatory burden of the brain. In turn, this neuroinflammation can impair the regulatory processes of the brain and cause pathological changes, which could contribute to the increased prevalence of mood impairments in those with T2DM (Maraldi et al., 2007).

This mechanism can also have effects on cognitive performance through structural damage to neuronal cells. Structural features of neurons can be affected because the glycosylation of myelin protein (protein that forms a multi-layer membrane around nerve cell axons to increase neuronal signal speed) alters its antigenicity leading to an infiltration of monocytes, macrophages and neutrophils and subsequent activation of microglia. This results in increased secretion of pro-inflammatory cytokines that can increase nerve excitability and neuroinflammation (Benatti et al., 2016). In support of this, previous research has found an accumulation of microglia in the hippocampus to be accompanied by elevated levels of the pro-inflammatory cytokines IL- 1β and TNF-α in diabetic rats (Hwang et al., 2014). In human studies, the presence of T2DM has been linked with elevated levels of IL- 1β and TNF-α (Chen et al., 2007; Boni-Schnetzler et al., 2008; Kowluru & Odenbach, 2004). Further increases in these pro-inflammatory cytokines have been detected when T2DM coexists with obesity (Cieslak, Wojtczak & Cieslak, 2015). Taken together, these findings may partially explain the relationship between T2DM and cognitive impairment.

To summarise, dysfunction of the BBB endothelium (see Section 2.4.1.1) can increase BBB permeability. The increase in BBB permeability allows plasma components, immune molecules and cells to invade the brain and activate resident microglia. As a response, primed microglia increase production of pro-inflammatory cytokines which ultimately leads to

neuroinflammation. This neuroinflammation has many negative connotations such as damaging neuronal cell axons (reducing signal speed) and apoptosis (cell death). In humans, the presence of T2DM has been associated with elevated levels of pro-inflammatory cytokines (Chen et al., 2007; Boni-Schnetzler et al., 2008; Kowluru & Odenbach, 2004) whilst animal models have identified an accompanying accumulation of microglia in the hippocampus of diabetic rats (Hwang et al., 2014). This suggests that the increased cognitive deficits seen in those with T2DM may be due to neuroinflammation of the relevant brain region. This mechanism may also explain the link between poorer glucose tolerance and negative mood outcomes as a higher neuroinflammatory burden may cause pathological changes in the brain. This may impair regulatory processes and result in the higher rate of negative mood and depression seen in those with T2DM (Maraldi et al., 2007).

2.4.3 Neurotransmitters

The dysfunction of glucose transport across the BBB (see Section 2.4.1.1) can also have negative connotations for the synthesis and regulation of some neurotransmitters. A dysregulation of neurotransmitters can offer a partial explanation for the relationship between glucose tolerance, cognitive performance and mood (Awad et al., 2004). For example, the synthesis of acetylcholine (ACh) is dependent upon an influx of its' building block acetyl coenzyme A (acetyl-CoA), which is obtained from glucose (Blass & Gibson, 1979; Gibson et al., 1978). Previous research has indicated that a deficit in ACh is associated with cognitive impairment (Rush, 1988; Rusted & Warburton, 1989). Given that glucose is required for optimal ACh synthesis, it is plausible that a dysfunction in glucose transfer across the BBB may result in reduced ACh availability and subsequent cognitive deficits. Animal models have supported this by identifying an association between T2DM and reduced ACh production in rodents (Welsh & Wecker, 1991). Furthermore, increasing the availability of ACh through peripheral glucose injections has been reported to improve memory in rats (Messier et al., 1990; Ragozzino et al., 1996; 1998). It has been suggested that this mechanism may explain why the glucose facilitation effect is more prominent in those with poorer glucose tolerance (Lamport et al., 2009). The researchers propose that those with poorer glucose tolerance are more likely to have deficits in ACh availability, making increases in ACh synthesis (through glucose administration/consumption) more beneficial to cognitive performance.

The dysfunction of neurotransmitters may also explain the relationship between poor glucose tolerance and mood disorders. For example, insulin resistance has been shown to attenuate insulin-induced excitability in dopaminergic neurons, resulting in decreased dopamine signalling and activity (Kleinridders et al., 2015; Konner et al., 2011). Indeed, streptozotocin-induced diabetes in rats has also been shown to decrease brain dopamine synthesis rates (Trulson & Himmel, 1983). Structural damage to the dopaminergic neurons from an increased neuroinflammatory burden can also reduce dopamine release and signal transduction (see Section 2.4.1.2). Previous post-mortem research comparing suicide victims to healthy individuals reported reduced concentrations of dopamine in the brain regions that mediate mood, such as the amygdala, in the suicide group (Klimeck et al., 2002). Furthermore, several neuroimaging studies support the hypothesis that major depression is associated with a

reduction in dopamine secretion and activity (D'Haenen & Bossuyt, 1994; Ebert et al., 1996; Shah et al., 1997). Taken together, the findings indicate that the prevalence of negative mood outcomes, observed in those with poorer glucose tolerance may be partially explained by an underlying dysfunction of neurotransmitters such as dopamine.

2.4.4 The HPA axis and glucocorticoids

The HPA axis is a major neuroendocrine system that involves direct influences and feedback interactions between the hypothalamus, pituitary gland and the adrenal glands. It is considered the common mechanism that mediates an organism's stress response by regulating the release of glucocorticoids such as cortisol and corticosterone (Malenka, Nestler & Hyman, 2009). Interestingly, increases in insulin resistance, characteristic of T2DM, have been associated with hyperactivity of the HPA axis and subsequently elevated levels of glucocorticoids (Bruehl et al., 2007; Chiodini et al., 2007; Godoy-Matos et al., 2006; Joseph et al., 2015; Raff & Magill, 2016; Reagan et al., 2008; Roy, Collier & Roy, 1990). Previous research has also indicated that elevated glucocorticoid levels are associated with reductions in neural glucose uptake (de Leon et al., 1997; Hornet et al., 1990; Sapolsky, 1986) as well as reduced synaptic plasticity and neurogenesis (Alfarez et al., 2003; Kerr et al., 1989; McEwen, 2000; Pavlides et al., 1993; Stanahan et al., 2008). It has been demonstrated that acute and chronic rises in glucocorticoids (e.g. cortisol) can impair cognitive performance in areas such as attention, episodic and spatial memory (Aisa et al., 2007, 2008; Horner, 1990; Sandstrom et al., 2011; Wolf, 2003). Therefore, elevated levels of glucocorticoids such as cortisol may offer a potential mechanism connecting cognitive impairment and poor glucose tolerance. Although, it should be noted that the underlying mechanisms that associate abnormal glucose tolerance and HPA axis dysfunction are unknown (Chan et al., 2005; Reagan et al., 2008).

Interestingly, cortisol has also been associated with insulin regulation (Convit, 2005). Previous research has indicated that an increase in cortisol levels can lead to increased insulin resistance (Phillips et al., 1998; Plat et al, 1996; Rizza, Mandarino & Gerich, 1982) and reduced insulin transport across the BBB (Baura et al., 1996; Laron, 2009). It has been demonstrated that the hippocampus contains the largest concentrations of both cortisol and insulin receptors within the brain (Convit, 2005) and that an acute rise of cortisol is associated with reduced hippocampal glucose metabolism (de Leon et al., 1997). This suggests that the hippocampus and its' involvement in cognitive domains such as memory is susceptible to increased levels of circulating glucocorticoids. As the presence of poorer glucose tolerance has been associated with HPA axis hyperactivity, it is plausible that those with T2DM are more likely to display hippocampal injury (van Harten et al., 2006). This is supported by previous research that identifies an association between T2DM and hippocampal atrophy (Bruehl et al., 2009; Gold et al., 2007; den Heijer et al., 2003; Korf et al., 2006). Given that hippocampal atrophy is linked with memory impairment (Manschot et al., 2006; Wrighten et al., 2009), this would explain why episodic memory deficits are often reported in those with T2DM (Boyle et al., 2018; Sunram-Lea & Owen, 2017).

HPA axis dysfunction may also explain the increased negative affect displayed by those with poorer glucose tolerance. Previous research has found elevated levels of circulating cortisol to

be associated with negative mood outcomes and increased risk of affective disorders such as bipolar disorder and depression (Ellenbogen et al., 2004, 2010; Handley et al., 1980; Van Eck et al., 1996). It has also been demonstrated that primates, humans and rodents that have suffered previous life traumas display HPA axis hyperactivity (Heim et al., 2008; Heim & Nemeroff, 2002; Sanchez et al., 2001). Taken together, these findings suggest that HPA axis hyperactivity may not be a result of major depression but rather a persistent neurobiological abnormality that predisposes an individual to depression (Pariante & Lightman, 2008). Therefore, it appears that poor glucose tolerance and negative mood share a bi-directional relationship via induced hyperactivity of the HPA axis.

2.4.5 The role of insulin

Increased insulin resistance is a hallmark of glucose tolerance abnormalities such as T2DM. It is known that insulin receptors exist in the brain and that insulin is absorbed by neural areas (Banks et al., 1997). The highest concentration of insulin receptors has been previously reported to occur on the cell walls of the hippocampus (Craft & Watson, 2004; Dore et al., 1997; Marks et al., 1990; Messier, 2004). Therefore, it is likely that insulin has a significant impact on the relationship between glucose tolerance and cognitive performance (Craft et al., 1993). Previous research has indicated that insulin resistance within the brain can result in neurons becoming insulin deficient (Strachan, 2003). Ultimately, this results in a reduced neuronal glucose uptake via insulin-facilitated diffusion. Furthermore, the acute administration of insulin has been shown to improve cognitive performance (Baker et al., 2003; Benedict et al., 2004; Craft et al., 1996, 2003; Kern et al., 2001; Reger et al., 2006; Watson et al., 2003). Additionally, improved insulin sensitivity via thiazolidinediones (TZD) administration has also been shown to improve cognitive performance (Ryan et al., 2004; Watson & Craft, 2004). This suggests that insulin resistance (and its' effects on neuronal glucose uptake) may be a moderating factor in the relationship between glycaemic response, glucose tolerance and cognition.

Given the link between insulin and neuronal glucose uptake, it is plausible that increased insulin resistance may lead to cognitive deficits (Geroldi et al., 2005; Messier & Tutenberg, 2005; Stranahan et al., 2008). Although there is no current evidence that poor glucose tolerance directly leads insulin resistance in the brain, there is support for an association between diabetic states and decreased insulin transport across the BBB (Banks et al., 1997; Baskin et al., 1985; Kaiyala et al., 2000). As aforementioned, it is known that some regions of the brain contain insulin sensitive glucose transporters (GLUT4 & GLUT8), particularly the hippocampus (Ashfari et al., 2017; Convit, 2005; Reagan et al., 2002). Therefore, a reduced amount of insulin reaching the brain via impaired BBB transport could reduce neuronal glucose uptake (Awad et al., 2004; Choeiri et al., 2002; Reagan et al., 2002). Furthermore, it is also known that insulin aids in the storing of glycogen in the brain (Brown et al., 2002; Gailliot, 2008). Glycogen is the stored form of glucose, and blood glucose levels are maintained at the expense of glycogen stores in fasted individuals (Wasserman, 2009). It has also been demonstrated that acute insulin administration can restore glycogen stores in hyperglycaemic and diabetic rodents (Daniel et al, 1977; Nahas & Abdul-Ghani, 1989; Thurston et al., 1975).

This suggests that insulin resistance, synonymous with T2DM, could result in depleted glycogen stores in the brain, leading to a reduced ability to synthesise glucose during cognitive demand (Brown, 2004; Gruetter et al., 2003).

Interestingly, insulin resistance has been associated with accelerated biological ageing of cells through the increased production of Reactive Oxygen Species (ROS) (Smith et al., 1995; Roriz-Filho, 2009). Subsequently, an increase in oxidative stress has been demonstrated to augment the brain's neuroinflammatory burden and the risk of apoptosis (Benatti et al., 2016). There is also evidence that heightened insulin levels within the brain are associated with beta-amyloid and tau protein mis-folding (Farris et al., 2003; Roriz-Filho, 2009). The mis-folding of these proteins can result in amyloid plagues, loss of neuronal structural integrity, and subsequent neuronal apoptosis (Haass & Selkoe, 2007; Nussbaum, Seward & Bloom, 2013; Pulawski et al., 2012). An elevated accumulation of mis-folded beta-amyloid and tau proteins has been reported in the brains of Alzheimer and diabetic patients (Hamley, 2012, Sims-Robinson et al., 2010). Insulin levels within the brain are also associated with neurotransmitters such as gamma-aminobutyric acid (GABA), N-methyl-D-aspartate (NMDA) and epinephrine (Guy et al., 2005; Kopf & Baratti, 1999; Mooradian, 1997). For example, epinephrine (also known as adrenaline) is involved in the "fight or flight" response by increasing blood flow and heart output among other roles (Bell, 2009; Khurana, 2008). Research has indicated that epinephrine release, during stressful stimuli, induces insulin resistance in the cell tissues of humans and rodents (Deibert & Defronzo, 1980; Chiasson et al., 1981; Porte, 1967; Porte et al., 1966; Shikama & Ui, 1975; Rizza et al., 1980). As aforementioned, insulin resistance can reduce insulin transport into the brain and subsequently result in reduced insulin-mediated glucose uptake by neurons (Awad et al., 2004; Banks et al., 1997; Baskin et al., 1985; Choeiri et al., 2002; Kaiyala et al., 2000; Reagan et al., 2002). Given that those with T2DM already experience insulin resistance, any epinephrine-induced reductions in insulin sensitivity may further reduce neuronal glucose uptake. Taken together, these findings indicate that increased insulin levels may negatively impact cognition through neuronal cell damage, whilst insulin resistance may impair cognitive performance by limiting insulin-mediated glucose uptake within the brain.

2.4.6 Chronic hyperglycaemia

Chronic hyperglycaemia (high glucose concentrations) is characteristic of poorer glucose tolerance observed in conditions such as T2DM. Previous research has indicated that frequent and extended periods of hyperglycaemia can adversely affect the structural integrity of the brain, leading to subsequent cognitive impairment (Arvanitakis et al., 2004; Lam et al., 1991; Perantie et al., 2007; Salim et al., 2009; Wrighten et al., 2009). The current evidence suggests that hyperglycaemia affects cognition through multiple mechanisms (Roriz-Filho et al., 2009). Firstly, an increased production of Advanced Glycation End-products (AGEs) has been reported during periods of hyperglycaemia and those with poorer glycaemic control (Goldin et al., 2006; Jakus & Rietbrock, 2004). Hyperglycaemia has also been linked to an increased production of Reactive Oxygen Species (ROS), which are by-products of energy metabolism (Devasagayam et al., 2004; Hayyan, Hashim & AlNashef, 2016). The two processes have been found to result in

both micro-vascular damage and brain atrophy (Gold et al., 2005; White et al., 2002; Yan et al., 2003), with the extent of hyperglycaemia being associated with increased complications (Jakus & Rietbrock, 2004). Furthermore, there appears to be a bi-directional relationship between AGEs and ROS, with increases in one leading to subsequent increases in the other (Wrighten et al., 2009; Yan et al., 2003; Yao & Brownlee, 2010). This suggests that chronic hyperglycaemia could accommodate an unremitting cycle of damage to the brain between AGEs and ROS, which may affect cognition. Indeed, animal models have demonstrated a link between increased ROS accumulation in the hippocampus with memory impairment (Fukui et al., 2001; Nicolle et al., 2001). As aforementioned (see Section 2.4.1.1-2.4.1.2), increased oxidative stress can increase BBB permeability resulting in an increased neuroinflammatory burden (Benatti et al., 2016). Higher levels of pro-inflammatory cytokines and primed microglia have been reported in the brain and blood of those with T2DM (Boni-Schnetzler et al., 2008; Chen et al., 2007; Hivert et al., 2009; Zunszain et al., 2011). Taken together, the findings provide evidence that chronic hyperglycaemia can affect cognition by increasing AGEs and ROS production. These two processes then cause significant neural damage, which can adversely affect cognitive performance and mood.

Interestingly, AGEs affect nearly every type of cell in the body and are considered to be an important factor in the biological aging process (Glenn & Stitt, 2009; Semba et al., 2009; Vistoli et al., 2013). An accumulation of AGEs has been associated with older age (Wrighten et al., 2009). Given that hyperglycaemia can increase the production of AGEs, this supports the suggestion that T2DM can augment neural aging (Biessels et al., 2008). Therefore, this would indicate that presence of T2DM can be more deleterious for cognitive performance in older adults compared to younger ones (Awad et al., 2004; Lamport et al., 2009). Indeed, recent reviews have highlighted that older participants tend to display greater cognitive impairments, which are amplified further when poorer glucose tolerance is present (Boyle et al., 2018; Sunram-Lea & Owen, 2017). To conclude, it appears that chronic hyperglycaemia can have adverse effects on cognition through increased AGEs production and oxidative stress. These two processes have deleterious effects on micro-vascular networks and neural regions, which result in subsequent functional impairment. Such effects appear amplified with increasing age, meaning that abnormal glucose tolerance would be particularly damaging from a cognitive aspect in older adults.

2.4.7 Summary of mechanisms

There are likely multiple mechanisms that underlie the relationship between glycaemic response, glucose tolerance, cognitive performance and mood. These largely focus on consequences of poorer glucose tolerance such as loss of BBB integrity, altered glucose and insulin availability, dysregulation of neurotransmitters, elevated AGEs and ROS production, as well as neuronal damage and apoptosis. All of these mechanisms can have an effect on cognitive performance and mood by altering the functional capacity of various brain regions. It has also been demonstrated that older age is associated with cognitive impairment, potentially through a naturally higher accumulation of AGEs. This suggests that the presence of poorer glucose tolerance in older adults can be particularly deleterious to cognitive

performance. It is likely that cognitive impairment is the result of the aforementioned mechanisms operating in a cascade-like fashion. For example, increased oxidative stress (brought about by chronic hyperglycaemia) leads to loss of BBB integrity and subsequent neuroinflammation. In turn, areas like the hippocampus may be adversely affected by the increased neuroinflammatory burden, leading to memory impairments. Thus, it appears that a combination of the aforementioned mechanisms occurring concomitantly is the likely moderator of cognitive impairment in those with poorer glucose tolerance.

2.5 Summary of glycaemic response, glucose tolerance, cognitive performance and mood

This chapter has reviewed and summarised previous research relating to the aims of this thesis. There is some evidence that the consumption of LGI/LGL foods can be beneficial for the glycaemic response and cognitive performance in both adults and children, although conflicting results have been found in both groups. Whilst meal consumption appears to be associated with more positive affect compared to meal omission conditions, the comparison between LGI/LGL and HGI/HGL conditions is limited and the current findings have provided minimal differences in mood outcome measures. Interestingly, glucose tolerance appears to be a major factor in cognitive performance and subjective mood, with poorer glucose tolerance often being associated with poorer cognitive performance and increased negative mood outcomes. A review of potential underlying mechanisms suggests that poorer glucose tolerance is often associated with an increasingly deleterious multifactorial combination of physiological processes (Section 2.4). The ultimate outcome of these processes is a loss of neuronal structure and functionality as well as dysregulation of vital hormones and neurotransmitters. Ultimately, this harmful combination of the aforementioned mechanisms leads to cognitive impairment.

Upon review, it is clear that there is a distinct lack of acute studies that have extended the testing paradigm beyond a single meal, with a large focus on the breakfast meal. Surprisingly, the majority of studies did not match energy and macronutrient content between glycaemic conditions, which may have confounding effects on the glycaemic response and subsequent cognition. Inconsistencies in glycaemic testing were also evident with large variations in frequency and assessment times being identified between studies. Finally, there appears to be a distinct lack of acute studies which have investigated both cognitive performance and subjective mood differences between GI/GL conditions in T2DM samples. Therefore, it is clear that the current single meal testing paradigm needs extending to the investigation of a fully representative day's diet that is matched in energy and macronutrient content between conditions. The timing of glycaemic and cognitive assessments should be carefully considered during such investigations. Finally, it would be of interest to compare glycaemic and cognitive outcome measures within healthy and T2DM samples (i.e. comparatively good and poor regulators within each sample) and between these samples. Such comparisons would provide further insight into the potentially moderating role of glucose tolerance in the relationship between glycaemic response and cognition. These issues are addressed by the subsequent series of studies presented in this thesis.

Chapter 3

Materials & Methods

This chapter provides detailed information and rationale on the methodology and testing materials used throughout this thesis.

3.1 Glycaemic measures

As displayed in Chapter 2, the majority of research investigating the association between GI/GL and cognition measures glucose concentrations via finger prick capillary samples. The one exception to this was Brindal et al., (2012) who implemented a continuous glucose monitoring system (CGM). The main difference between these two methods is the source of their glucose readings. Specifically, the finger prick method returns blood glucose concentrations whereas CGM readings provide the glucose levels in the interstitial fluid (ISF) i.e. the fluid that surrounds the cells in the body (Cengiz & Tamborlane, 2009). Findings from previous research have shown glucose concentrations in the brain to be positively associated with glucose levels in both the blood (Rostami & Bellander, 2011) and subcutaneous ISF (Nielsen et al., 2005).

Research comparing the implementation of both methods has reported a time delay between measurements, with ISF glucose levels falling in advance of blood plasma glucose concentration (Maggs, 1996; Maggs et al., 1995; Sternberg et al., 1996; Thome-Duret et al., 1996). However, a review of this time delay concluded that the error component between blood and ISF glucose readings (<6%) was not a significant obstacle in the advancement of CGM use (Rebrin & Steil, 2000). More recently, it was found that a CGM system can provide reliable readings accurate to blood glucose concentrations if calibrated during a steady state such as fasting when blood and ISF glucose levels are most similar (Chlup et al., 2015). The main advantage of using a CGM system rather than finger pricks is that it allows for a greater number of data points to be obtained over a longer time frame (Surman & Fleeman, 2013). The use of a CGM system also has the advantage of allowing a participant to carry out cognitive tasks without being disturbed by finger pricks. How each method was implemented throughout this thesis is described below (sections 3.1.1-3.1.2).

3.1.1 Capillary blood finger prick sampling

In study 1, blood glucose levels were measured by taking capillary blood samples via finger pricks. Specifically, the Accu-Chek Aviva Blood Glucose Meter System was used (Roche Diagnostics, UK). All blood glucose measurements were in units of millimoles per litre (mmol/l). Capillary sampling guidelines set out by the World Health Organisation were followed (WHO, 2010). Prior to a sample being taken, a participants' fingertip was cleaned using an alcohol wipe. A disposable one-time use lancet was used to pierce the edge of a

participants' fingertip, where the blood was sampled. To minimise discomfort from successive finger pricks, different fingertips were used throughout a test day.

3.1.2 Continuous Glucose Monitoring (CGM)

During studies 2 and 3, glucose levels in the interstitial fluid were measured using a FreeStyle Libre continuous glucose monitoring system (Abbott Diabetes Care Inc., UK). At the beginning of a test day, a participant would have the glucose sensor attached to the back of their upper arm, which would self-calibrate over the course of an hour. The sensor would automatically measure interstitial glucose levels every minute and store readings at 15-minute intervals for 8 hours. This data would wirelessly transmit to the reader held by the experimenter when the sensor was scanned. At the end of a test day, the sensor was removed from the participants' arm by the researcher and disposed of. The use of CGM caused minimal discomfort to participants, with little to no pain being reported during both application and removal of sensors.

Previous research has reported interstitial glucose measurements with the FreeStyle Libre system are accurate compared with capillary blood glucose reference values and remain accurate over 14 days of wear in type 1 and 2 diabetics (Bailey et al., 2015). The experimenters also reported that sensor accuracy was not affected by individual factors such as BMI, age or type of diabetes. The mean delay time between FreeStyle sensor readings and blood glucose reference readings was found to be 4.5±4.8 minutes, which is within the estimated 5-10 minute delay of interstitial glucose readings (Rebrin, Sheppard & Seil, 2010). The time delay of the FreeStyle Libre is also significantly lower than some older CGM sensors, which have been shown to have time delays up to 32 minutes (Mazze et al., 2009). Therefore, the FreeStyle Libre system was appropriate for implementation in this thesis due to the high level of reading accuracy, which is unaffected by varying individual factors, along with the short time delay between the systems interstitial glucose readings and reference blood glucose values.

3.1.3 Glucose Regulator Type

Where good and poor glucose regulators within a sample were identified (Chapters 4-6), a glucose tolerance composite score was calculated. Previous reviews have suggested that the use of a glucose tolerance composite score that incorporates a number of glucose tolerance parameters would offer greater ecological validity rather than focusing on a single parameter (Lamport et al., 2009). This is a sensible approach considering that a single measurement or parameter is unlikely to be the most accurate predictor of an entire postprandial glycaemic response, and furthermore, different parameters are associated with different mechanisms. For example, the rate of absorption in the gut may be reflected by the initial rise in glucose concentration, whereas insulin sensitivity may be reflected by the speed of decline towards the end of a glycaemic response. The timing of cognitive assessments related to the glycaemic response can influence the association shown between glucose tolerance parameters and cognition. For example, the strong relationship between peak glucose and cognitive

performance in studies of participants with normal glucose tolerance (for review see Lamport et al. 2009) may be due to examining cognition immediately after glucose consumption during the initial rise of the glycaemic response. In the context of the studies presented in this thesis, multiple cognitive assessments occurred throughout the day during different phases of the glycaemic response. Therefore, it was appropriate that a combination of glucose tolerance parameters was included when calculating a glucose tolerance composite score for participants.

Specifically, the glucose tolerance composite score used throughout this thesis consisted of four glucose tolerance parameters; baseline glucose, peak glucose, positive incremental area under the curve (iAUC) and total area under the curve (tAUC) (both AUCs were calculated using the trapezoidal method, which is defined further in Appendix A). These four parameters were calculated for each condition, producing a total of eight separate values for each participant. Initially, the mean and standard deviation for each parameter value within a condition was calculated. Following this, participant z scores were produced for each individual parameter value. The resulting eight z scores for each participant were then averaged to produce a single mean z score per participant. A median of all participant mean z scores was then calculated. Any participant with a mean z score below the median was labelled as a good glucose regulator, whereas a participant with a mean z score above the median was labelled as a poor glucose regulator.

Each parameter used to calculate the glucose tolerance composite scores throughout this thesis was selected by considering its' relation to glucose tolerance and the glycaemic response, along with the potential underlying mechanisms. Firstly, glucose concentration at baseline (fasting) was included as this parameter is an indicator of glucose tolerance status currently used in the diagnosis of T2DM (WHO, 2016). Given the link between higher baseline glucose levels and poorer glucose tolerance, this parameter largely reflects the insulin secretion and sensitivity of the participant at the time of testing (WHO, 1999, 2006, 2016). Previous research has found an association between baseline glucose levels and cognition, with higher baseline glucose concentrations being correlated with poorer cognitive performance (Convit et al., 2003; Rolandsson et al., 2008). The peak glucose concentration value was also included as this parameter indicates the rate of gastric emptying and intestinal glucose absorption following meal consumption (Gonlachanvit et al., 2003; Rayner et al., 2001). Several cognitive studies have reported a correlation between peak glucose values and cognition, with higher peak values being associated with poorer cognitive performance, particularly on memory tasks (Awad et al., 2002; Donohoe & Benton, 2000; Messier et al., 2003). The final two parameters included in the glucose tolerance composite score were positive iAUC and tAUC. Both parameters were included as they consider different aspects of the glycaemic response during calculation of AUC. As shown in Figure 3.1, the positive iAUC method calculates all positive areas above the baseline glucose line, whereas the tAUC method calculates the entire area below all observed glucose concentrations (Cardoso et al., 2011). Due to their calculation methods, positive iAUC is considered to more accurately describe the glycaemic rising potential of a meal, whereas tAUC is a descriptive factor of the entire glycaemic response (Cardoso et al., 2011; Le Floch et al., 1990). As both methods consider the rise and fall of glucose concentration, the underlying mechanisms reflected include gastric emptying, intestinal glucose absorption rates and insulin sensitivity. The benefit

of also including the tAUC method is that it considers hypoglycaemic events in relation to baseline glucose values (Le Floch et al., 1990). Cognitive research has demonstrated that higher values of iAUC and tAUC are associated with poorer cognitive performance (Convit et al., 2003; Kaplan et al., 2000).

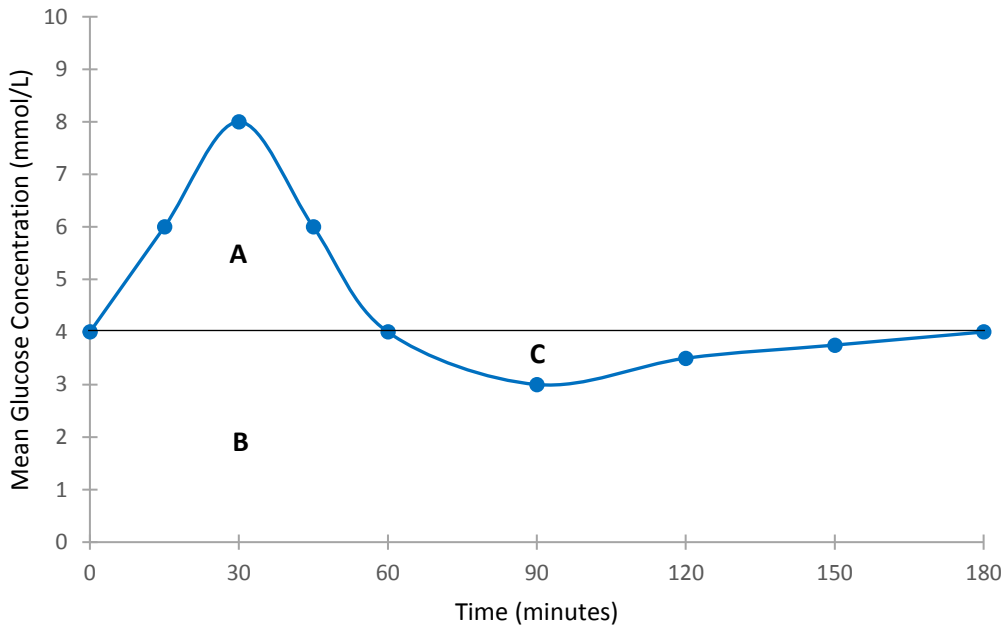


Figure 3.1: Graphical representation of positive iAUC and tAUC methods

Values represent hypothetical glucose concentration data points. The black line represents the baseline (fasting) glucose concentration. Positive incremental area = A. Total area = A + B.

3.2 Cognitive measures

3.2.1 Task selection

The cognitive task battery that was administered in studies 2 and 3 comprised of four computerised tasks each programmed with E-Prime 2.0 (Psychology Software Tool, Inc). Collectively, the tasks examined psychomotor function, sustained attention, executive function and working memory. Psychomotor function was selected as impairment in this cognitive domain has been associated with both type 1 and type 2 diabetes (Ryan et al., 1992; Ryan, 2005; Ryan & Geckle, 2000). However, the acute effects of GI/GL manipulation on psychomotor function in healthy participants appears to be under-investigated compared to other cognitive functions such as attention and memory, making it an interesting area to explore further with a multiple-meal paradigm in samples with varying glucose tolerance (Philippou & Constantinou, 2014; Adolphus et al., 2016). Executive function and working memory were examined as previous research suggests that these cognitive domains are particularly sensitive to GI manipulation during the postprandial phase (See Chapter 2). Finally, attention was assessed as this cognitive domain shows sensitivity in the postprandial phase,

which could be explored further using a multiple-meal paradigm rather than previous single meal testing conditions (see Chapter 2). All implemented tests were able to be administered repeatedly over a test day with minimal interference from previous versions or carry over effects. For example, later performances on a word recall based episodic memory task may have been affected by the learning of words in previous versions, therefore the decision was made not to include an assessment of verbal memory. Full descriptions of each implemented task are provided below.

3.2.2 Choice reaction time task (CRT)

This task is a measure of general alertness and psychomotor speed. The cognitive functions required for this task have been associated with bilateral frontoparietal network activation, including ventral (PMv) and dorsal (PMd) premotor areas (Cisek & Kalaska, 2005; Davare et al., 2006; Hoshi & Tanji, 2004; Perfetti et al., 2010). Throughout the task, participants were provided with a fixation 'x' in the centre of a computer screen. The fixation 'x' was in Courier New font and had a font size of 24. For each trial, the participants were required to indicate whether a target 'x' had appeared to the left or right of the fixation point by pressing the relevant key (z or m) as quickly as possible. The inter-stimulus interval (ISI) that separated the end of one trial from the beginning of the next ranged from 250ms to 1500ms in 250ms segments during the task. Each ISI was randomly selected by E-prime 2.0 from a pre-designated list in between each trial. This task lasted for approximately 3 minutes with a total of 60 targets presented. The dependent variables were accuracy score (out of 60) and mean reaction time (ms) (for correct responses).

3.2.3 Rapid visual information processing task (RVIP)

The RVIP task is a measure of sustained attention and working memory, which have been found to correlate with activation of both right and left frontoparietal networks (Coull et al., 1996; Lawrence et al., 2003; Neale et al., 2015). During this task, participants were presented with a continuous string of single numerical digits ranging from 1 to 9 in the centre of a computer screen. The string of numbers was presented at a rate of 75 digits per minute, with each trial fixed at 800ms. Participants continuously monitored the digits for two specific target strings, which were '1, 3, 5' and '6, 4, 2'. When a target string was observed, the participant would indicate this by pressing the space bar as quickly as possible. This task lasted approximately 4 minutes with a total of 270 single digits being presented, including 20 target strings. The dependent variables for the task were accuracy score (out of 20) and mean reaction time (ms) (for correct responses).

3.2.4 Merged CRT & RVIP task (Merged)

The merged task reported here was a novel concept, designed to increase cognitive effort by combining the testing parameters of the CRT and RVIP tasks. Thus, this task is a measure of

sustained attention, working memory and psychomotor speed. By combining two simpler tests into a more difficult task the effect of increasing task difficulty can be explored, as previous studies have shown that glucose enhancement is most likely for more difficult tasks (Kennedy & Scholey, 2000; Korol & Gold, 1998; Meikle, Riby & Stollery, 2005). Throughout this task, each trial presented to the participant contained two aspects; (1) a single digit in the centre of the computer screen, and (2) a target 'x' that appeared to the left or right of the central digit. Both aspects continuously changed between trials in a pseudorandom fashion. The participant was required to press the relevant key (z or m) to indicate which side the 'x' had appeared on every trial, and simultaneously press the space bar if either target string (1, 3, 5 or 6, 4, 2) was observed. Each trial had a fixed duration of 800ms, and the inter-stimulus interval (ISI) that separated the end of one trial from the beginning of the next ranged from 200ms to 1000ms in 200ms segments. Each ISI was randomly selected by E-prime 2.0 from a pre-designated list in between each trial. This task lasted approximately 8 minutes with a total of 270 trials being presented, including 250 CRT-only trials and 20 combined task trials. The dependent variables for the task were accuracy scores on combined task trials (out of 20) as well as mean reaction time (ms) (for correct responses). To allow for comparison between the CRT task and the Merged task, CRT aspect performance was also measured during the Merged task. The first outcome variable for this was accuracy score, defined as correctly identifying the side 'x' had appeared regardless of whether a target number sequence was present (out of 270 trials). The second outcome variable was mean reaction time (ms) (for correct responses).

3.2.5 Letter memory task (LM)

The letter memory task is a measure of executive function, which has been associated with neural activation in the prefrontal and parietal cortices (Funahashi, 2001; Funahashi et al., 2013; Miller & Cohen, 2001; Petersen & Posner, 2012; Stoet & Snyder, 2009). During this task, participants were presented with a series of letters (consonant letters only, no vowel letters were included), which appeared individually in the centre of a computer screen. The number of letters presented was either 5 or 7 (8 of each), which randomly varied throughout each task run. When a sequence of letters had ended, participants were presented with a screen that displayed four options. Participants had to press the relevant button (1, 2, 3 or 4) to indicate which option contained the last four letters that had appeared. Once the participant had indicated their choice, the next sequence of letters would begin. The series of letters were presented at a rate of 30 letters per minute, with each letter appearing for 2000ms. At the end of each sequence, the participant had a maximum of 8000ms to indicate their choice of the four options presented. If they made no choice during the 8000ms, the next sequence would begin and no selection was recorded. This task lasted for approximately 5 minutes with a total of 96 letters being presented across 16 separate sequences. The dependent variables for the task were accuracy score (out of 16) and mean reaction time (ms) (for correct responses).

3.2.6 Alternate forms & counterbalancing

Alternate forms of all cognitive tests were used at each presentation. For the CRT, RVIP and Merged tasks, new forms were randomly generated within E-prime 2.0 each time a task was run. Due to the complexity of the Letter Memory task, alternate forms were pre-programmed and presented to participants for each task run. During each cognitive task battery run, the order of cognitive task presentation was also counterbalanced to further minimise potential order effects from the participants. This methodology was implemented as previous research has reported order effects when using a cognitive task battery (Collie et al., 2003). To ensure appropriate counterbalancing, Williams matrices were used (Williams, 1949). The counterbalanced order of task administration for each study where cognitive performance was assessed (Chapter 5-6) can be found in Appendix B.

3.2.7 Global Cognitive Measures

In order to investigate overall cognitive performance across tasks, three global cognitive measures were calculated retrospectively; Global Cognitive Accuracy (GCA), Global Cognitive Reaction Time (GCRT) and Global Cognitive Performance (GCP). Each of the global cognitive measures represented a mean Z score value for each participant across every session (see calculation process below). GCA represents the mean Z score calculated from the four task accuracy components, whereas GCRT is the mean Z score derived from the four task reaction time components. GCP represents the mean Z score calculated from the four accuracy and four reaction time components.

Global cognitive measures were obtained using the following process; (i) all baseline and testing session means (both conditions) for an individual task variable (e.g. CRT accuracy) from all participants were placed in a singular column within SPSS Statistics 24, (ii) Z scores were then calculated with these data (iii) If the individual task variable involved a reaction time component the resulting Z score for each session was multiplied by -1, so that a higher Z score represented a faster reaction time, (iv) The resulting Z scores for each individual participant were then averaged to produce an average score for Accuracy (mean of four accuracy components), Reaction Time (mean of four reaction time components) and Cognitive Performance (mean of all accuracy and reaction time components) at each baseline and testing session, (v) Finally, Z scores were then calculated with these average scores to produce each individual's Global Accuracy, Global Reaction Time and Global Performance values at every session. Thus, an individual with a Global Z score above 0 at a particular session performed better than the sample average. Whilst, an individual with a Global Z score below 0 at any given session performed worse than the sample average. This allowed performance on a given Global measure to be not only be compared within, but also across, sessions.

3.3 Subjective measures

3.3.1 Subjective Mood

The Bond-Lader mood questionnaire (Bond & Lader, 1974) was administered during all studies. The questionnaire presents participants with 16 individual lines, with each line having opposing mood related adjectives at either end (see Appendix C). To indicate their current mood in relation to the adjectives, the participant would mark each line with a pen nearest the adjective that represented their feelings at the present moment. Each line had a length of 100mm, resulting in a recordable score of 0 to 100 for each pairing of mood related adjectives. Weighted scores for adjective pairings were then combined according to published criteria (Bond & Lader, 1974), resulting in three mood sub-factors; alertness, anxiety and contentment. Higher ratings in these sub-factors indicated higher levels of alertness, anxiety or contentment.

3.3.2 Subjective Hunger, Fullness and Sleepiness (HFS)

Previous research has found that a meal containing complex CHOs, typically found in low GI foods, is associated with a lower perception of fatigue and a higher degree of satiety compared to a simple CHO meal (Pasman et al., 2003). To compare the effects that the two meal profiles had on satiety and fatigue, subjective measurements of hunger, fullness and sleepiness were recorded during testing. The three factors were presented as visual analogue scales in an identical manner to the Bond-Lader mood scales (see section 3.7.1). Higher self-reported ratings indicated higher subjective levels of hunger, fullness or sleepiness (see Appendix D).

3.4 Testing procedure

Screening and testing for all studies were carried out in the Hugh Sinclair Unit at the University of Reading. Screening involved the measurement of height, weight, BMI, blood pressure (see below for method) and fasting glucose levels (see section 3.1.1 for method). If the study involved cognitive performance assessment (chapters 5-7), then a familiarisation run of the cognitive task battery was also administered at screening (see section 3.2 for cognitive task battery details). The dates of both test visits were confirmed at the end of the screening session and were separated by a minimum of 7 days and a maximum of 30 days. The order that a participant took part in the two experimental conditions was determined with an online researcher randomiser (Urbaniak & Plous, 2013). During each study, all glycaemic measures and cognitive assessments were completed in an individual testing room to minimise potential distractions. All meals were prepared and served by the researcher in testing kitchen in the Hugh Sinclair Unit. Participants were required to consume every meal within 15 minutes (Jenkins et al., 1980). Strict adherence to testing time points was achieved through the use of digital timers throughout all studies. Specific timings of glycaemic, cognitive and mood assessments are detailed in each study chapter. The order in which participants completed glycaemic conditions for each study can be found in Appendix E.

3.4.1 Body mass index (BMI)

The weight (kg), height (m) and BMI (kg/m^2) for every participant was measured with a Tanita BC-418MA body composition monitor (TANITA corporation, Tokyo). Initially, participants were asked to stand bare foot on two electrode plates, whilst holding two electrodes in their hands. The monitor used Bioelectric Impedance Analysis (BIA) technology to send a very low electrical signal through the feet and hands to the legs, arms and abdominal area. This signal passes through water in hydrated muscle tissue quickly but meets resistance when it hits fat tissue. This resistance is measured and input into Tanita equations to calculate body composition measurements. The Tanita used the following equation to calculate an individuals' body mass index (BMI);

$$BMI = \frac{weight\ (kg)}{\big(height(m)\big)^2}$$

3.4.2 Blood pressure (BP)

Systolic and diastolic blood pressure readings were measured with a validated Omron M3 digital blood pressure monitor (Omron Corporation, Kyoto). Three blood pressure readings were taken, separated by intervals lasting 2 minutes as per standard laboratory protocol. All measurements were recorded with the participant sitting on a chair. The blood pressure cuff was placed around the left arm of the participant, which was resting on the arm of the chair.

3.5 Data analysis

Data processing and analysis was carried out using Microsoft Office Excel 2007, E-DataAid 2.0 and IBM SPSS Statistics 24.

3.5.1 Outlier procedures

Before full statistical analyses were run, all data was screened for potential outliers using IBM SPSS Statistics 24 in accordance with a published data cleaning protocol (Tabachnick & Fidell, 2013) and is described below. For all analyses that involved a reaction time (RT) component, only the RT values of correct responses were included. Any RT values below 100 milliseconds were deemed to be reactions carried over from previous trials or anticipatory responses and were removed (Odom et al., 2004; Oram & Perrett, 1992; Rolls & Tovee, 1994). Z scores for all remaining RT data points were then calculated (i.e. a Z score was calculated for each response by every participant across all sessions), and RTs with a z score of 3.29 or greater were identified as potential outliers. Traditionally, the chance of sampling a z score of 3.29 is <0.001, meaning that such occurrences suggest an extreme outlier (Tabachnick & Fidell, 2013). During this process, histograms and box plots were also consulted in order to better understand potential outliers within the scale (time frame) of each task. If a potential outlier had a Z score

of 3.29 or greater but was deemed to be within a reasonable and justifiable distance of the sample scores, it was not removed (as discussed in the relevant study chapter). Individual Z scores were calculated using a participant's score (x_i), the sample mean ($\bar{x}$) and sample standard deviation (SD) in the following equation:

$$Z = \frac{\mathrm{Xi} - \bar{\mathrm{x}}}{SD}$$

After this procedure, mean RTs for each participant at each test session were then calculated. For the RVIP and Merged task, the number of false positives per session (max = 250) was calculated and converted into a percentage (i.e. number of false positives/250 X 100). Any session where the percent of false positives was 33% or higher was removed from analysis on all other elements (RT and accuracy score). Given that participants knew to search for three-digit target strings throughout both tasks but did not know the maximum number of targets available, a 33% cut off represents the maximum percentage of false positives a participant could produce if they simply indicated a choice every three digits and were consistently incorrect. Therefore, anything above this 33% cut off was deemed as guessing or loss of attention and was removed from analysis (outlier removals are described in the relevant study chapter).

3.5.2 Repeated measures data

All repeated measures data were analysed using Linear Mixed-effects Models (LMMs). LMMs use a complex linear regression procedure to model variance that is related to both fixed parameters (e.g. conditions with varied GI) and random parameters (e.g. individual differences between participants) within multiple layers of the same model (Field, 2009; Hoffman & Rovine, 2007). Previous comparisons between LMM and repeated ANOVA have identified numerous advantages of implementing LMM analysis (Field, 2013; Hoffman & Rovine, 2007; Magezi, 2015; Shek & Ma, 2011). Firstly, LMM analysis does not require the assumption of independence between data observations, which is generally not the case in a repeated setting. Without this requirement the LMM analysis can more accurately model the covariance structure of data, thereby increasing analysis power. Furthermore, LMM analysis does not require balanced data meaning that a participant with missing data points can be still be included, further increasing power of analysis. Finally, there is no requirement for dependent variables to be normally distributed, as is the case with all regression analysis. Although regression analysis assumes normal distribution of regression residuals, previous research has reported that the distribution of residuals does not influence LMM outcomes (Gelman & Hill, 2007). The use of LMM analysis also means that repeated covariates can be included within the model, which is not possible using repeated ANOVA.

To achieve consistency between analyses, LMMs have been systematically applied throughout this thesis. As shown in Table 3.1, the independent variables of Condition, Time and Regulator Type were entered as fixed factors in the LMM analysis. The covariates of Gender, Age, BMI, Baseline Glucose and Baseline Outcome Variable were also included as fixed factors.

Participant ID was included in the LMM analysis as a random factor to control for non-independence of data within participants (Sweet & Grace-Martin, 2011).

Table 3.1: The Linear Mixed Model used for analysis of outcome variables

Fixed Factors	Interactions	Random Factors
Condition (IV)	Condition*Time	Participant ID (COV)
Time (IV)	Condition*Regulator Type	
Regulator Type (IV)	Time*Regulator Type	
Gender (COV)	Condition*Time*Regulator Type	
Age (COV)		
BMI (COV)		
Baseline Glucose (COV)		
Baseline *Outcome Variable* (COV)		

*IV = Independent Variable, COV = Covariate

Additionally, the Time factor was also specified as a repeated variable to control the covariance structure for each participant. This was necessary as LMM analysis models the covariance structure using a covariance matrix of all repeated data observations. Within the covariance matrix, variances are defined along the diagonal positions, whereas covariances are defined along off-diagonal positions. It is possible to select and impose different covariance structures within the covariance matrix. The matrix that generally suits all data is the unstructured matrix, which assumes covariances are unpredictable and do not have any underlying fixed structure. However, a limitation of the unstructured matrix is that a small number of participants and many repeated time points can lead to insufficient degrees of freedom being available to determine a solution for the model. In such cases, another covariance structure can be applied. Although, it is important to note alternative covariance structures do make some assumptions about the variances and covariances within the matrix. In the context of this thesis, the most appropriate alternative covariance structure was the heterogenous, autoregressive structure (ARH1). The ARH1 matrix assumes that variances are heterogenous, while covariances increase with greater proximity within the matrix (i.e. adjacent repeated measurements are more closely correlated than measurements recorded a greater time apart). Where possible, an unstructured covariance matrix was applied. In the cases where a solution for the model could not be found, the ARH1 matrix was implemented. LMM analysis produces a measure known as a '-2 log linear' value (-2LL), which is proportional to the variance unaccounted for by the model. Thus, a smaller -2LL value indicates a better fit of the LMM to the data. These values were considered so that the best available model was used in all analyses throughout this thesis.

3.5.3 Reporting of statistical results

The terminology used throughout this thesis is consistent with LMM analysis. For LMM analysis, factors are described as significant or non-significant predictors of dependent variables, whereas ANOVA outcomes are reported as main effects and interactions (Field,

2013). Beta values (i.e. regression coefficients) have been included for all covariates that were found to be significant in predicting outcome variables. If a fixed factor or interaction was found to be significant, pairwise comparisons were consulted to interpret the findings. Bonferroni corrections were applied to all post hoc tests as this correction method gives the most control over type 1 error (Field, 2009).

Throughout this thesis, only significant findings ($p<0.05$) and findings trending towards significance ($p<0.1$) have been fully reported in the experimental chapters (Chapters 4-6). Full LMM results for each chapter have been tabulated in Appendix F.

Chapter 4

The Effect of Glycaemic Index Variation on Glycaemic Response and Mood in Healthy Participants Across the Day (Study 1)

4.1 Introduction

Varying the GI of foods and meal is an effective strategy for manipulating the glycaemic response (Jenkins et al, 1981; Wolever at al., 1988). Typically, a low GI food will produce a lower glycaemic response compared to a high GI food (Jenkins et al, 1981). Furthermore, the GI of a meal can affect the glycaemic response shown at the following meal, even after an overnight fast. This finding is known as the second meal effect (Wolever at al., 1988). The link between glycaemic response and cognition is well established, with poor glycaemic control often being associated with an increased prevalence of cognitive deficits (Awad et al., 2004; Lamport et al., 2009). To investigate any potential cognitive benefits of improved postprandial glycaemia, many studies have implemented GI as a way of manipulating the glycaemic response whilst measuring cognitive outcomes. Although this research has returned mixed results (see Chapter 2, Section 2.2), the current evidence generally favours low GI meals for improved cognitive performance, noticeably in areas of memory and attention (Blaak et al., 2012; Boyle et al., 2018; Philippou & Constantinou, 2014). However, previous acute studies investigating glycaemic response and cognitive performance are predominantly limited to a single meal testing paradigm, with a large majority focusing on breakfast, whilst a number of methodological issues such as the meals not being matched for calories or macronutrient content exists in the current literature (see Chapter 2). Such methodological discrepancies make it difficult to attribute any cognitive differences to solely different glycaemic responses produced by GI variation between testing conditions.

Interestingly, the meals implemented in some studies have failed to produce divergent glucose response profiles (e.g. Smith & Foster, 2008). Considering that humans consume multiple mixed meals on a daily basis, the current singular meal testing paradigm used in acute studies warrants extension to an examination of any potential glycaemic and cognitive differences over a longer time period, where multiple meals can be consumed. Before any cognitive tests can be implemented, it is important to first ensure that test meals do produce varied glycaemic responses between GI conditions. Therefore, the primary aim of this study was to design two meal profiles which show measurable differences in glycaemic response over the course of the day. To address this aim, the concept of GI was applied (Wolever et al., 1991). Each condition was labelled on the basis of the glycaemic profile it was expected to produce. Specifically, the Favourable Glycaemic Profile (FGP) condition consisted of three LGI meals, whereas the Unfavourable Glycaemic Profile (UGP) condition consisted of the three HGI meals. In theory, the FGP condition would be expected to produce a lower glycaemic response across the day when compared to the UGP condition. The glycaemic profiles produced by these two conditions were compared. Meals were isocaloric and matched for macronutrients.

Research investigating the acute effects of GI on mood states has returned mixed results (see Chapter 2, Section 2.3) with some studies finding no differences between single meal GI/GL conditions (Cooper et al., 2015), whilst others report higher levels of happiness and alertness

following a LGI meal (Micha et al., 2011). Interestingly, one previous study found potential evidence for a second meal mood effect, with participants displaying lower levels of confusion 140 minutes after lunch if they consumed a LGI, rather than a HGI breakfast (Benton & Nabb, 2004). This finding suggests that the GI of one meal can have an acute effect on subjective mood ratings after consumption of the following meal. As humans have multiple meals throughout the day, the glycaemic response to two GI meal profiles and any potential mood effects warrant investigation. Therefore, the second aim of the present study was to investigate whether the FGP and UGP have different effects on subjective mood throughout the day. Subjective hunger, fullness and sleepiness were also measured at each mood assessment to consider their potential confounding effects on subjective mood ratings (Hill, Weaver & Blundell, 1991; Holt et al., 1999; Lo et al., 2016; Macht & Dettmer, 2006, Parker, Parker, & Brotchie, 2006).

An individual's glucose tolerance largely determines their postprandial glycaemic response. Specifically, a person with good glucose tolerance will display a lower glycaemic response compared to an individual with poor glucose tolerance if they were to consume the same meal (Wolever & Brand-Miller, 1995). Typically, a poor glucose regulator will suffer from more glycaemic variability i.e. more frequent peaks and troughs in glucose concentration. There is a wealth of literature investigating the association between glucose tolerance and cognition, with many reporting increased cognitive impairment in those with poorer glucose tolerance (Awad et al., 2004; Cukierman et al, 2005; Geijselaers et al, 2015; Messier, 2005). Similarly, an association between glucose tolerance and mood has been identified, with poorer glucose regulators displaying worsened mood and higher levels of depression relative to good glucose regulators (Maraldi et al., 2007; Nabb & Benton, 2006). Research has also found that long term improvements in glucose tolerance are associated with a sustained improvement in mood (Lustman et al., 2007). Given the link between glucose tolerance and mood, the third aim of the present study was to investigate whether individual glucose tolerance status will affect the potential relationship between glycaemic response and subjective mood ratings following consumption of the two experimental conditions (FGP vs. UGP). To address this, the sample was split into good and poor glucose regulators using a glucose composite score (as described in Section 4.2).

4.1.1 Summary of aims

Aim 1: To design two meal profiles which show measurable differences in glycaemic response over the course of the day.

Aim 2: To investigate whether any significant differences in subjective mood ratings are found between these two glycaemic response conditions (FGP vs. UGP).

Aim 3: To investigate whether glucose tolerance status within a sample predicts subjective mood ratings.

4.1.2 Study Hypotheses

Hypothesis 1: The consumption of low GI meals (FGP condition) will be associated with an improved glycaemic response profile across the day compared to the consumption of high GI meals (UGP condition).

Hypothesis 2: The FGP condition will be associated with improved subjective mood ratings across the day compared to the UGP condition.

Hypothesis 3: Good glucose regulators will display significantly better subjective mood across the day compared to poor glucose regulators within the sample.

4.2 Method

4.2.1 Power analysis

A prior power analysis was conducted using Gpower 3.1 to determine the sample size required. Assuming an effect size of d = 0.78 (Kaur et al., 2015) with a statistical power of 0.95 and an alpha level of 0.05, 24 participants were deemed sufficient to detect glycaemic differences between conditions. Extra participants were recruited in the event of a drop out to ensure minimum sample size requirements were achieved.

4.2.2 Recruitment

Opportunistic sampling involved emails to relevant group mailing lists, the handing out of fliers and posters, as well as posting on the University of Reading campus and Reading town notice boards. All participants were recruited from the local population within the county of Berkshire.

4.2.3 Participants

Twenty-four healthy, normal weight adults (6 males and 18 females) were recruited with a mean age of 38.4 years (SD = 15.3) (see section 4.3.1 for full details). Inclusion criteria were aged between 18–65 years old and a BMI of 18.5-25kg/m^2. All participants were self-reported healthy non-smokers, with no relevant food intolerances or allergies.

Exclusion criteria were a medical diagnosis of high blood cholesterol or pressure, any condition that could affect glucose metabolism (e.g. diabetes, anaemia and pregnancy), or a disease of complication which affected their thyroid, heart, brain, vascular system, bones, kidneys, gastrointestinal tract, respiratory system or liver. Prescribed anti-depressants was also an exclusion criterion as these drugs have the potential to impact glucose metabolism (Deuschle, 2013; Himmerich, Minkwitz & Kirkby, 2015) and cognitive function (Hindmarch, Kimber & Cockle, 2000; Knegtering, Ejick & Huijsman, 1994). Finally, any participant that was a self-reported professional athlete was excluded, as these individuals could have improved glucose

uptake due to skeletal muscular adaptations such as increased GLUT4 glucose transporter expression (Goodyear & Kahn, 1998).

4.2.4 Study design

The present study was conducted using a counterbalanced, randomised, crossover design with two experimental conditions. The two conditions were carried out in a counterbalanced or to minimise the potential of confounding order effects. The order in which a participant took part in the experimental conditions was obtained by entering participant numbers into an online researcher randomiser (Urbaniak & Plous, 2013). To allow for potential drop outs, twice the number of required participants was entered into the randomiser prior to the study. This resulted in half of the sample beginning with the FGP condition, whilst the other half took part in the UGP condition initially. For the order of participation see Appendix E. The two conditions were (i) Favourable Glycaemic Profile (FGP) - a LGI diet consisting of a breakfast, lunch and afternoon snack and (ii) Unfavourable Glycaemic Profile (UGP) - a HGI diet consisting of a breakfast, lunch and afternoon snack. The independent variables were Condition, Time and Regulator Type, whilst the dependent variables were Glycaemic Response and Subjective Mood, Hunger, Fullness and Sleepiness ratings.

4.2.5 Nutritional manipulations

Two novel meal profiles that vary in their GI values were designed using every day food products such that each meal was of representative content and size. Each meal profile consisted of three consecutive meals; breakfast, lunch and an afternoon snack. The GI of each food or drink that comprised a meal was obtained from published tables of GI values (Atkinson et al., 2008; Foster-Powell et al., 2002; Henry et al., 2005). The GI of a mixed meal is expressed as the weighted mean of its' component GI values, with the weighting of each component based on the proportion of carbohydrate provided (Wolever & Jenkins, 1986; Wolever et al., 1991). When conducting cognitive research that implements test meals, it is vital that each meal is matched for energy and macronutrients between conditions. This methodology ensures that any cognitive performance differences seen between experimental conditions cannot be explained by these potential confounding variables. Both meal profiles were isocaloric (1,310kcal), with each meal being matched for calories and macronutrients between conditions. For both conditions, the test meals were administered at 09:00, 12:00, and 15:00 to mimic representative meal times found in a habitual diet (see Figure 4.1).

4.2.5.1 Favourable Glycaemic Profile (FGP)

The FGP meal profile contained three meals (breakfast, lunch and snack) designed to have a low GI value (<55). This diet had an average GI value of 33.7 and an average GL value of 20.5. According to the Foster-Powell et al. (2002) method the GL of the day's diet was 61.5. This diet was implemented to produce a low glycaemic response after each meal leading to less

glycaemic variability throughout the day. The nutritional content and a detailed breakdown of macronutrients for each meal can be found in Table 4.1.

4.2.5.2 Unfavourable Glycaemic Profile (UGP)

The UGP meal profile contained the same three meals; breakfast, lunch and a snack. In this condition the meals had a high GI value (>70). This diet had an average GI value of 79.4 and an average GL value of 52. According to the Foster-Powell et al. (2002) method the GL of the day's diet was 156.1. This diet was designed to produce a high glycaemic response following the consumption of each meal, with the aim of producing greater glycaemic variability throughout the day. The nutritional content and a detailed breakdown of macronutrients for each meal can be found in Table 4.2.

Table 4.1: Nutritional composition of the FGP condition meal profile.

FGP breakfast	Weight (g)	Fat (g)	Protein (g)	CHO (g)	Energy (kcal)	Fibre (g)	GI	PCF (%)	GI * PCF/100	GI * CHO/100
All Bran Cereal	29	1.02	4.06	13.92	96.86	7.83	44	26.5	11.7	6.1
Skimmed Milk	126	0.13	4.28	6.3	44.1	0	48	12	5.8	3
Apple Juice	226	0.23	0	26.44	106.22	0.23	40	50.3	20.1	10.6
Yoghurt	84	1.18	4.2	5.88	51.24	0	35	11.2	3.9	2.1
Total	**465**	**2.56**	**12.54**	**52.54**	**298.42**	**8.06**		**GI**	**41.5**	**GL 21.7**
FGP lunch										
Pasta Bake	440	25.9	23.7	87.5	699.6	10.1	23	100	23	20.1
Total	**440**	**25.9**	**23.7**	**87.5**	**699.6**	**10.1**		**GI**	**23**	**GL 20.1**
FGP snack										
Raw Apple	133	0.13	0.53	15.69	70.49	2.39	32	29.2	9.3	5
Cashew Nuts	17	8.21	3.33	4.08	104.72	0.56	27	7.6	2.1	1.1
Apple Juice	290	0.29	0	33.93	136.3	0.29	40	63.2	25.3	13.6
Total	**440**	**8.63**	**3.86**	**53.7**	**311.51**	**3.24**		**GI**	**36.6**	**GL 19.7**

*CHO = Carbohydrate content (g), TMC = Total Meal Carbohydrate (g), PCF = CHO/TMC*100 = Proportion of Carbohydrate from each Food. GI values are taken from Foster Powell et al. (2002), Henry et al. (2005) and Atkinson et al. (2008).

Table 4.2: Nutritional composition of the UGP condition meal profile.

UGP breakfast	Weight (g)	Fat (g)	Protein (g)	CHO (g)	Energy (kcal)	Fibre (g)	GI	PCF (%)	GI * PCF/100	GI * CHO/100
Corns Flakes	30	0.27	2.1	25.2	113.4	0.9	93	47.3	44	23.4
Skimmed Milk	220	0.22	7.48	11	77	0.91	48	20.6	9.9	5.3
White Bread	38	0.65	3.31	16.95	88.54	0.02	75	31.8	23.9	12.7
Flora spread	3	2.1	0.02	0.02	18.93	0	0	0.3	0	0
Total	**291**	**3.24**	**12.91**	**53.17**	**297.83**	**1.83**		**GI**	**77.8**	**GL 41.4**
UGP lunch										
White Bread	76	1.29	6.61	33.9	177.08	1.82	75	38.3	28.7	25.4
Philadelphia spread	79	8.69	5.85	4.03	120.08	0.4	0	4.5	0	0
Cheddar cheese	46	16.05	11.68	0.05	191.36	0	0	0.2	0	0
Lettuce	40	0.2	0.32	0.68	6.4	0.36	15	0.7	0.1	0.1
Lucozade, original	293	0	0	49.81	205.1	0	95	56.3	53.5	47.3
Total	**534**	**26.23**	**24.46**	**88.47**	**700.02**	**2.58**		**GI**	**82.3**	**GL 72.8**
UGP snack										
Jelly beans	28	0.11	0.11	27.33	106.4	0	80	51.1	40.9	21.9
Lemon curd yoghurt	105	9.35	3.68	17.75	170.1	0.21	67	33.1	22.2	11.9
Lucozade, original	50	0	0	8.5	35	0	95	15.8	15	8.1
Total	**183**	**9.46**	**3.79**	**53.58**	**311.5**	**0.21**		**GI**	**78.1**	**GL 41.9**

*CHO = Carbohydrate content (g), TMC = Total Meal Carbohydrate (g), PCF = CHO/TMC*100 = Proportion of Carbohydrate from each Food. GI values are taken from Foster Powell et al. (2002), Henry et al. (2005) and Atkinson et al. (2008).

4.2.5.3 Food Preparation

All meals were prepared and administered by the researcher in the Hugh Sinclair Unit of Human Nutrition, University of Reading. All meals were served cold with the only exception being the FGP lunch meal, which was a microwaveable pasta bake. This meal was microwaved for the recommended amount of time, found on its' provided packaging. The required weight (g) of each food component was checked using a digital weighing scale, and the necessary volume (ml) of each drink component was confirmed using a measuring beaker. The experiment was conducted in a single blind manner, where the participants were not told the GI or GL values of the test meals. Participants were informed that the meals were matched for energy and macronutrients but were not told specific values concerning these areas. If participants asked for this information during testing, they were told they would receive it at the end of the study. This methodology minimised the risk of any subjective bias that the participant may display.

4.2.5.4 Washout

A minimum washout period of 7 days between visits was implemented (Lamport et al., 2011; Lamport et al., 2013). This length of time was deemed sufficient to ensure that consumption of one meal profile did not have an effect on the glycaemic response shown during the subsequent condition. A maximum period of 30 days was allowed between test visits, with any participant not abiding to these time restrictions being removed from the study. During the washout period, participants were instructed to eat their habitual diet and inform the researcher if their diet had dramatically changed for any reason.

4.2.6 Outcome variables

4.2.6.1 Glycaemic Response

Blood glucose was measured using the capillary finger prick sampling procedure detailed in Chapter 3, Section 3.1.1. During each testing session, a total of twenty-one finger prick capillary blood samples were taken. The assessment times for the samples were; immediately before each meal (0 minutes), and 15, 30, 45, 60, 90, and 120 minutes post meal consumption (See Figure 4.1). This procedure allowed glucose measurements to be taken regularly without interrupting meal consumption or subjective mood evaluations (see Figure 4.1 for test day procedure).

4.2.6.2 Self-report measures (questionnaires)

Subjective mood was measured using Bond & Lader Mood VAS (Bond & Lader, 1974). The outcome variables for this were scores of 0-100 for the three mood factors Alertness, Anxiety and Contentment. Hunger, Fullness and Sleepiness were measured using VAS, the outcome

variables for which were scores of 0-100. Full details of these can be found in Chapter 3. All subjective measures were assessed six times throughout the test day (see Figure 4.1).

4.2.7 Procedure

4.2.7.1 Screening

Potential participants who contacted the researcher were sent by email the study information sheet (Appendix G) and a health and lifestyle medical questionnaire (Appendix H) which they were required to read, complete and return before a screening session was arranged. This ensured that the participants met the inclusion criteria (see section 4.2.3 for inclusion and exclusion criteria). If a participant's response fulfilled the criteria, a one-hour screening session was arranged for the morning, at the University of Reading Hugh Sinclair Unit of Human Nutrition Research Unit. Before any measurements were taken, informed consent was obtained through the participant reading and signing an informed consent sheet (Appendix I). After obtaining informed consent; fasting glucose levels were measured by taking a finger-prick capillary sample with an Accu-Chek Aviva Blood Glucose Meter System (see Chapter 3 for method). Height, weight and BMI were measured using a Tanita BC-418MA body composition monitor whilst blood pressure readings were taken using an Omron M3 digital blood pressure monitor (see Chapter 3 for method). The screening form used by the experimenter can be found in Appendix J. Once screening was completed, the two test days were arranged, the first of which was required to be a least one week following the screening session.

4.2.7.2 Testing

4.2.7.2.1 Prior dietary instructions

Participants were asked to refrain from the consumption of alcohol and avoid any form of exercise for the 24 hours prior to a test visit. Participants were instructed to consume their evening meal prior to the day of testing between the hours of 6 and 8pm, and to keep this time consistent the evening before each test day. After the evening meal, participants were required to fast (no food or drink except water) for the rest of the day.

4.2.7.2.2 Day sessions

Participants were required to arrive at the Hugh Sinclair Unit of Human Nutrition in a fasted state from waking, having consumed nothing expect water on the morning of a test day. The schedule of a test day is shown in Figure 4.1. Upon arrival at 08:30 participants were given a short brief of the test day procedure in a quiet room where they were then able to watch television or read magazines provided. Testing began at 09:00 with a finger prick capillary sample immediately before the consumption of breakfast. Participants were required to consume the entire test meal within 15 minutes. A total of twenty-one glucose samples were taken throughout the day; immediately before each meal (0 minutes), and 15, 30, 45, 60, 90, and 120 minutes post meal consumption. Subjective Mood, Hunger, Fullness and Sleepiness

were self-evaluated at 15- and 105-minutes post meal consumption. Test days ended at 17:00 (see Figure 4.1). All procedures were in line with the Declaration of Helsinki (2014).

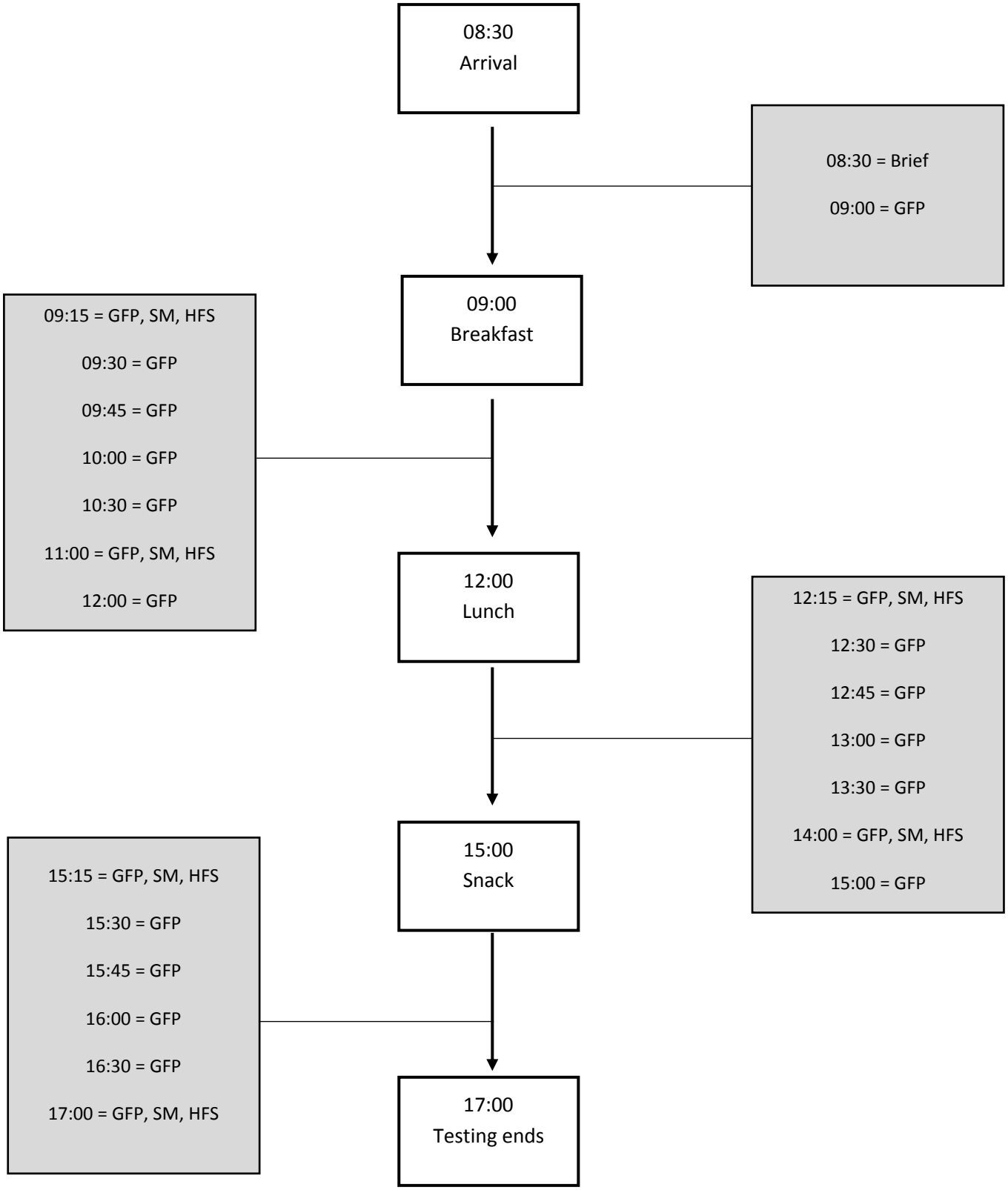


Figure 4.1: A flow diagram showing the step-by-step procedure for each test day

*GFP = Glucose Finger Prick sample, SM = Subjective Mood evaluation, HFS = Hunger, Fullness, Sleepiness evaluation.

4.2.8 Payment

The participant payment amounts were standardised in line with the Hugh Sinclair Unit of Human Nutrition guidelines. All participants received £50 per visit, giving a total of £100 paid to each. This amount was considered sufficient to financially remunerate participants for their time and any incurred travel expenses.

4.2.9 Ethical approval

This study received a favourable opinion from the School of Psychology Research Ethics Committee (SREC). Evidence of ethical approval can be found in Appendix J. The clinical trials ID number for this study is NCT03344185.

4.2.10 Statistical analysis

Linear Mixed Modelling was used to analyse the data, with the specific LMM presented in Chapter 3, Section 3.5.2 being carried out on each outcome variable (described below). Subject ID was included as a random effect in all models to control for non-independence of data within subjects (Sweet & Grace-Martin, 2011). Predictors of outcome variables and interactions of independent variables were only fully reported if statistically significant ($p<0.05$) or approaching significance ($p<0.1$). In both cases, pairwise comparisons were consulted and reported. All F values not reported in the results section are included in Appendix F.

The LMM consisted of Condition, Time and Regulator Type as the independent variables, which were entered as fixed factors. A participant who displayed a steadier glycaemic profile across the day would be considered a "better" glucose regulator compared to a participant who had greater peaks and troughs in their glycaemic profile. To label a participant as either a "Good" or "Poor" glucose regulator, a glucose composite score was implemented (see Chapter 3 for calculation method). The covariates of Gender, Age, BMI, Baseline Glucose, and Baseline Outcome Variable were also entered as fixed factors in the model. Baseline Glucose was defined as the first glucose reading taken on each test day. Similarly, Baseline Outcome Variable was defined as a participants' performance during the first session of each test day. Therefore, session 1 of each outcome variable became the baseline value entered into the model.

Overall, there were seven outcome variables for this study; Glycaemic Response, Alertness, Anxiety, Contentment, Hunger, Fullness and Sleepiness. The LMM described above was run on each outcome variable resulting in a total of seven models being performed during the present study analysis. Results deriving from the model are reported below for each outcome variable. For all significant findings, appropriate post hoc tests and bonferroni corrections were carried out and are also reported below.

4.3 Results

4.3.1 Participant characteristics

The characteristics of six male and eighteen female participants are shown in Table 4.3.

Table 4.3: Participant Characteristics (n = 24, 6 male, 18 female)

Variable	M	SD	Min	Max
Height (cm)	168	9.15	147	183
Weight (kg)	61.1	8.1	46.6	84.1
BMI (kg/m^2)	21.7	2.1	18.5	25
Age (years)	38.4	15.4	19	65
Fasting Glucose (mmol/L)	5.15	0.46	3.8	5.7

Note. Fasting Glucose value was obtained at screening.

*M = Mean, SD = Standard Deviation, Min = Minimum value, Max = Maximum value.

4.3.2 Glycaemic Response

An initial t test indicated that baseline glucose values were significantly higher in the FGP condition (M=5.19mmol/l, SD=0.49) compared to the UGP condition (M=5.02mmol/l, SD=0.09) at the start of the day; $t(23) = 2.209$, $p = 0.037$. As expected, the overall mean glucose concentration was significantly higher in the UGP condition (M=6.24 mmol/l, SD=0.24) compared to the FGP condition (M=5.94 mmol/l, SD=0.24), as indicated by Condition being a significant predictor $F(1, 195) = 18.214$, $p < 0.001$. The interaction between Condition and Time was also significant for Glycaemic Response, $F(19, 169) = 6.078$, $p < 0.001$. Bonferroni corrected post hoc tests revealed that the glycaemic response was significantly higher in the UGP condition at 45, 60, 90, 120, 195, 210, 225, 240, 270 and 420 minutes (all: $p<0.05$), which suggests participants experienced longer periods of hyperglycaemia after the consumption of HGI test meals compared to the LGI meals. However, the glycaemic response was significantly higher in the FGP condition at 15 minutes ($p=0.015$), which may reflect a slightly faster initial absorption of the FGP breakfast meal. The glycaemic response was also significantly lower in the UGP condition at 360 minutes ($p=0.001$) which is indicative of a reactive hypoglycaemia following a HGI meal. Finally, the higher glucose reading for the FGP at 375 minutes after snack consumption is simply due to the fact that the baseline prior to the snack was higher for the FGP, in actual fact the increase from pre-snack to post snack is greater for the UGP (see Figure 4.2).

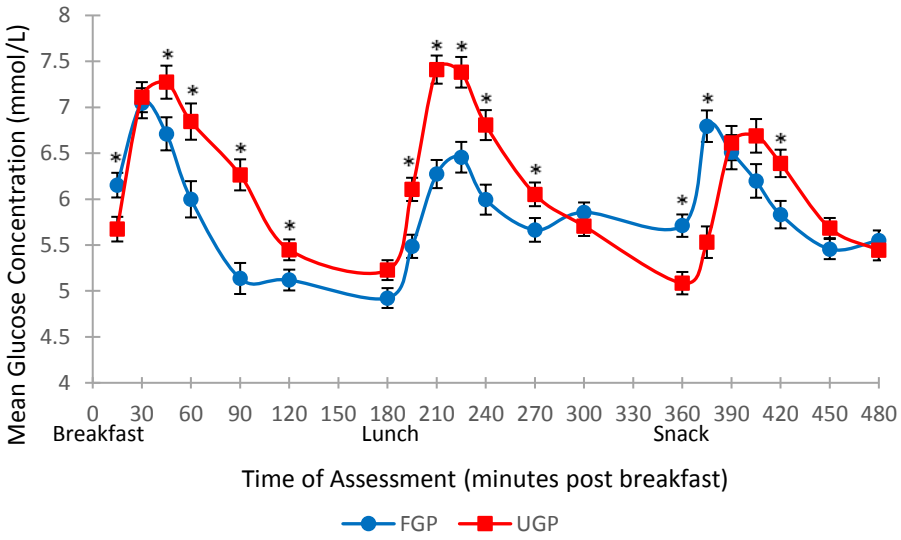


Figure 4.2: The interaction between Condition and Time for Glycaemic Response

Data points represent estimated marginal means across all participants at each time point. Error bars represent standard error of the mean. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Baseline glucose (0 minutes) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure shows that a significantly higher glycaemic response was observed in the UGP condition following each meal. However, a significantly higher glycaemic response in the FGP condition was observed 15 minutes after breakfast which may reflect a quicker absorption rate of the FGP breakfast. A significantly lower glycaemic response in the UGP condition is observed at 360 minutes, which is indicative of a postprandial dip following a HGI meal.

Overall, poor glucose regulators produced a significantly higher glycaemic response average (M=6.43mmol/L, SD=0.24) across the two conditions compared to good glucose regulators (M=5.75mmol/L, SD=0.24), as reflected by Regulator Type being a significant predictor, $F(1, 199) = 88.389$, $p < 0.001$. The interaction between Time and Regulator Type was also significant for Glycaemic Response, $F(19, 169) = 2.606$, $p = 0.001$, with post hoc tests showing that poor glucose regulators had significantly higher levels of glucose concentration at 30, 45, 60, 90, 120, 180, 195, 210, 225, 240, 270, 300, 375, 390, 405, and 420 minutes (all: $p<0.05$) (see Figure 4.3).

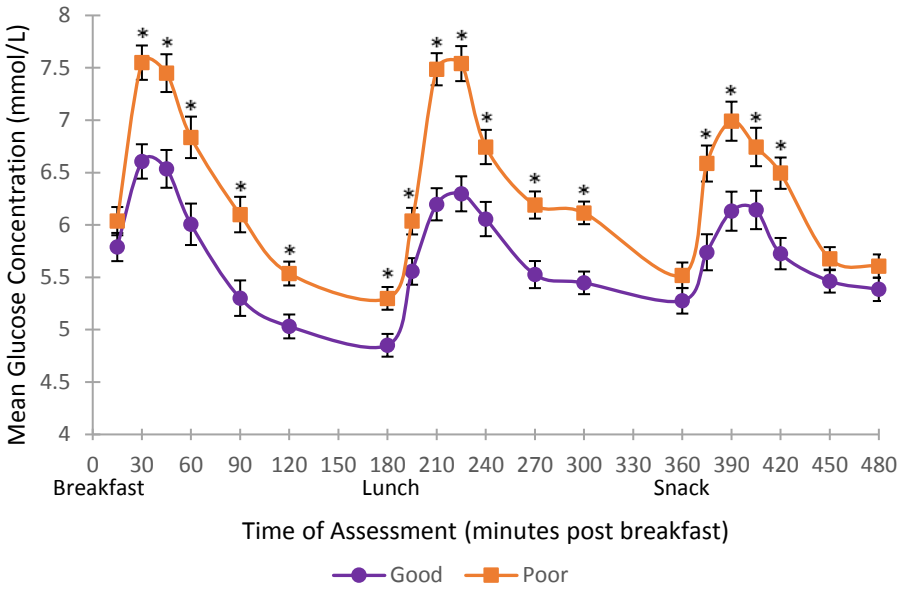


Figure 4.3: The interaction between Time and Regulator Type for Glycaemic Response

Data points represent estimated marginal means for good and poor glucose regulators at each time point across conditions (i.e. averaged across HGI and LGI meals). There were 12 good and 12 poor glucose regulators. Error bars represent standard error of the mean. An asterisk (*) indicates a significant difference between glucoregulatory groups at an assessment time point. Baseline glucose (0 minutes) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure indicates that the poor glucoregulatory group produced a significantly higher glycaemic response after the consumption of every meal.

The two-way interaction between Condition and Regulator Type approached significance, $F(1, 195) = 3.23$, $p=0.074$, which indicates that the difference between the good and poor regulators was smaller for the FGP condition, but nevertheless, for both conditions the response was still significantly higher for the poor regulators (see Figure 4.4).

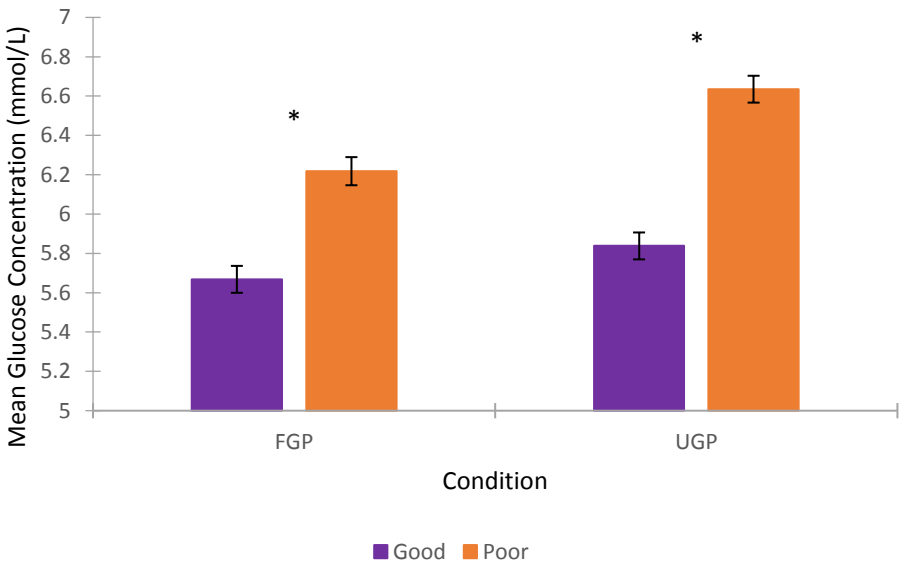


Figure 4.4: Glucose Concentration condition means for good and poor glucose regulators

Values represent estimated marginal means for good and poor regulators within each condition. There were 12 good and 12 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a condition. Error bars represent standard error of the mean. The figure shows that poor glucose regulators produced a significantly higher glycaemic response average in both conditions. A greater difference between glucoregulatory groups was observed in the UGP condition.

4.3.3 Subjective Mood

4.3.3.1 Alertness

The overall mean Subjective Alertness levels in the FGP condition (M=62.49, SD=11.48) and UGP condition (M=61.84, SD=11.48) did not significantly differ, as indicated by Condition being non-significant. Alertness significantly increased immediately after lunch consumption (relative to 11:00), and then dropped off, indicative of a post lunch dip, as indicated by post hoc tests following Time being a significant predictor, $F(4, 48) = 9.196$, $p < 0.001$ (see Figure 4.5). Furthermore, consumption of the afternoon snack greatly improved Subjective Alertness after the post lunch dip.

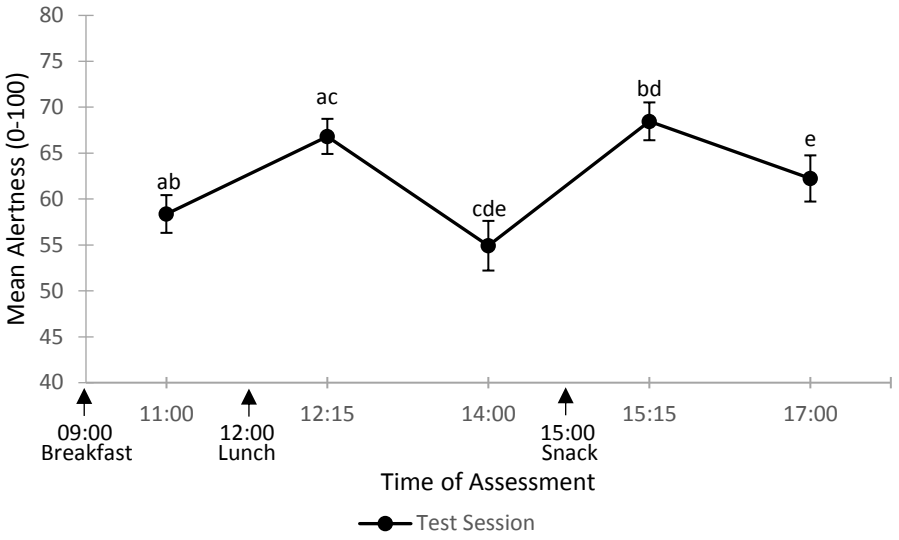


Figure 4.5: Mean Subjective Alertness for each testing session

Values represent estimated marginal means across all participants at each assessment time point across conditions. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. Baseline alertness (09:15) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure indicates that Subjective Alertness increased following meal consumption, but significantly dipped post lunch, indicative of a post-lunch dip.

4.3.3.2 Anxiety

The overall mean Subjective Anxiety levels in the FGP condition (M=30.29, SD=7.36) and UGP condition (M=31.95, SD=7.36) did not significantly differ, as indicated by Condition being non-significant. Interestingly, good glucose regulators (M=28.13, SD=7.56) reported significantly lower levels of overall Subjective Anxiety compared to poor glucose regulators (M=34.12, SD=7.56) as indicated by Regulator Type being a significant predictor, $F(1, 48) = 7.013$, $p = 0.011$. Neither Condition or Time were found to significantly predict Subjective Anxiety.

4.3.3.3 Contentment

The overall mean Subjective Contentment levels in the FGP condition (M=72.23, SD=7.01) and UGP condition (M=74.58, SD=7.01) did not significantly differ, as indicated by Condition being non-significant. However, the interaction between Condition and Regulator Type was significant, $F(1, 46) = 4.047$, $p = 0.05$. As shown in Figure 4.6, pairwise comparisons revealed that poor glucose regulators reported significantly higher subjective contentment levels during the UGP condition (M=76.77, SD=10.13) compared to the FGP condition (M=70.23, SD=10.33), whilst the opposite general pattern was observed for the good regulators.

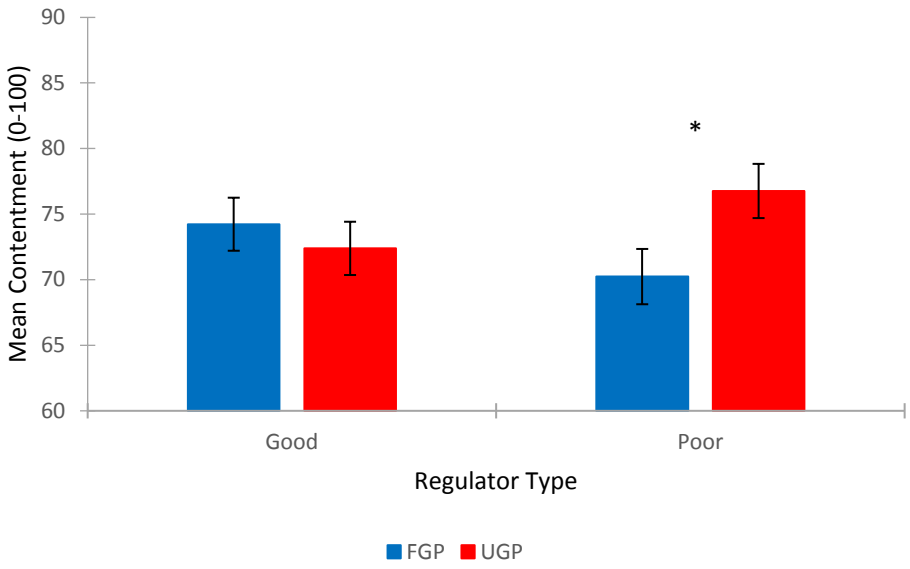


Figure 4.6: Subjective Contentment condition means for good and poor glucose regulators

Values represent estimated marginal means for good and poor regulators within each condition. There were 12 good and 12 poor glucose regulators. Data points with an asterisk (*) indicates significantly different condition means within a glucoregulatory group. Error bars represent standard error of the mean. The figure shows the poor glucose regulators were significantly more content in the UGP condition compared to the FGP condition, whilst the opposite general pattern is observed for the good glucoregulatory group.

4.3.4 Subjective Hunger, Fullness and Sleepiness (HFS)

4.3.4.1 Hunger

The overall mean Subjective Hunger levels in the FGP condition (M=34.81, SD=13.67) and UGP condition (M=33.88, SD=13.67) did not significantly differ, as indicated by Condition being non-significant. As expected, Subjective Hunger was significantly reduced following meal consumption, as indicated by post hoc tests following Time being a significant factor, $F(4, 48) = 20.413$, $p < 0.001$ (see Figure 4.7).

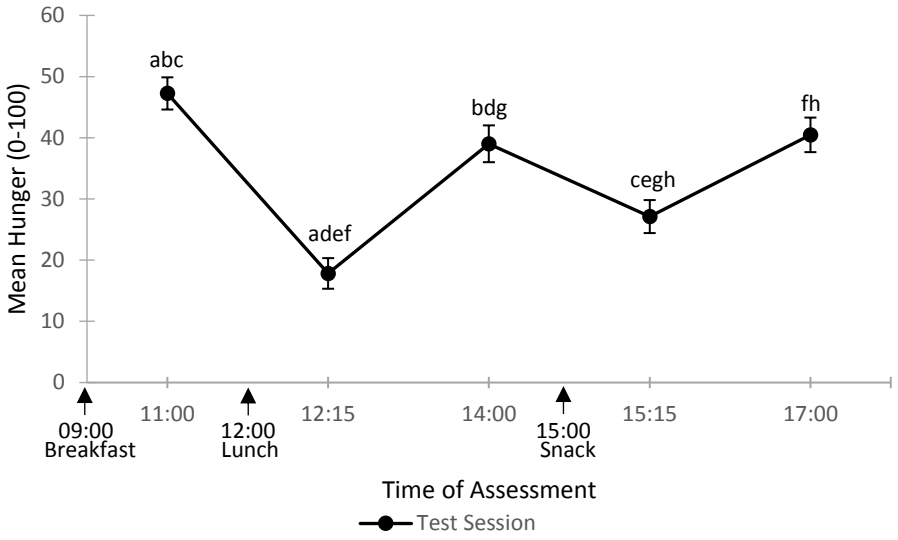


Figure 4.7: Mean Subjective Hunger for each testing session

Values represent estimated marginal means across all participants at each assessment time point across conditions. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. Baseline hunger (09:15) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure indicates that meal consumption led to reductions in Subjective Hunger, reflecting the expected satiating effect of the test meals.

The three-way interaction between Condition, Time and Regulator Type approached significance $F(4, 48) = 2.526$, $p=0.053$. As shown in Figure 4.8, poor glucose regulators were significantly hungrier at 12:15,15:15 and 17:00 in the UGP condition compared to the good glucose regulators, indicating that the UGP was not as effective at reducing hunger for the poor glucose regulators.

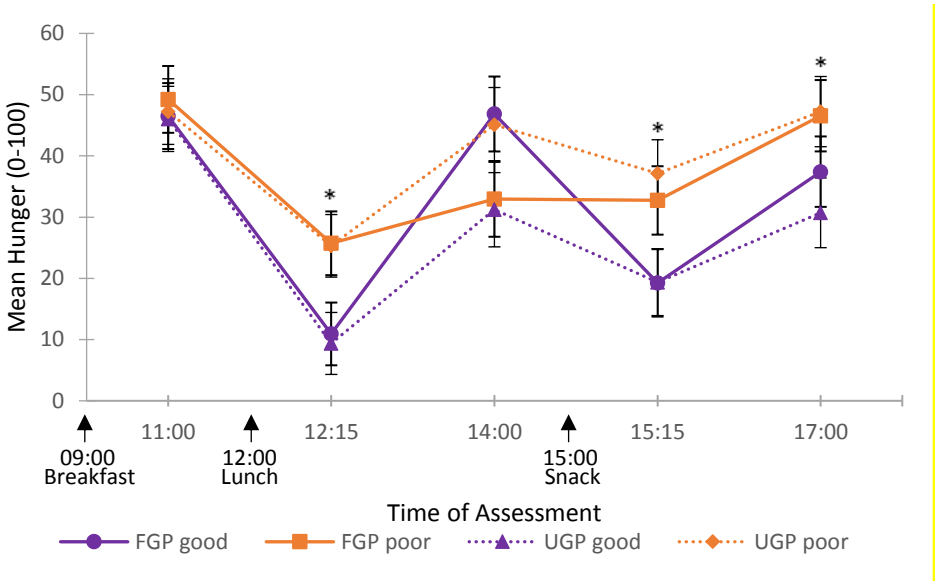


Figure 4.8: Subjective Hunger levels for good and poor glucose regulators within each condition

Values represent estimated marginal means for good and poor regulators at assessment time points within each condition (above = FGP, below = UGP). There were 12 good and 12 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators at an assessment time point within the UGP condition only. Black arrows represent meal consumption times. Error bars represent standard error of the mean. The figure shows that poor glucose regulators were significantly hungrier at 12:15, 15:15 and 17:00 in the UGP condition compared to good glucose regulators, which suggests the UGP condition was less effective at reducing hunger for the poor glucoregulatory group. No significant differences between glucoregulatory groups were found within the FGP condition.

Overall, the poor regulators (M=38.94, SD=14.2) were significantly hungrier than the good regulators (M=29.75, SD=14.2), as indicated by Regulator Type being a significant predictor, $F(1, 50) = 4.57$, $p = 0.037$. The Regulator Type by Time interaction; $F(4, 48) = 3.162$, $p = 0.022$, showed that this was due to differences post meal consumption, so the meals were more effective at reducing overall hunger for the good regulators (see Figure 4.9).

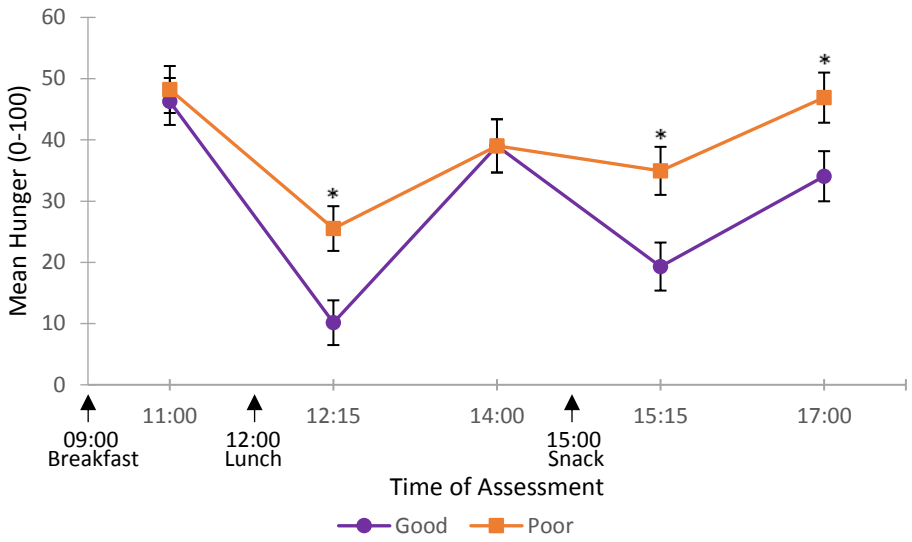


Figure 4.9: Mean Subjective Hunger for glucose regulators at each testing session

Values represent estimated marginal means for good and poor regulators at a test session across conditions. There were 12 good and 12 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators at an assessment time point. Black arrows represent meal consumption times. Error bars represent standard error of the mean. The figure indicates that good glucose regulators felt significantly less hunger following lunch and snack consumption, which suggests the meals were more effective at reducing subjective hunger for the good glucoregulatory group.

4.3.4.2 Fullness

The overall mean Subjective Fullness levels in the FGP condition (M=56.63, SD=21.43) and UGP condition (M=56.47, SD=20.76) did not significantly differ, as indicated by Condition being non-significant. As expected, Subjective Fullness was significantly higher post meal consumption (relative to pre-meal assessments), as indicated by post hoc tests following Time being a significant predictor, $F(4, 48) = 33.173$, $p < 0.001$ (see Figure 4.10). Neither Condition or Regulator Type were found to significantly predict Subjective Fullness, indicating that meal consumption increased fullness regardless of experimental condition or glucose tolerance status.

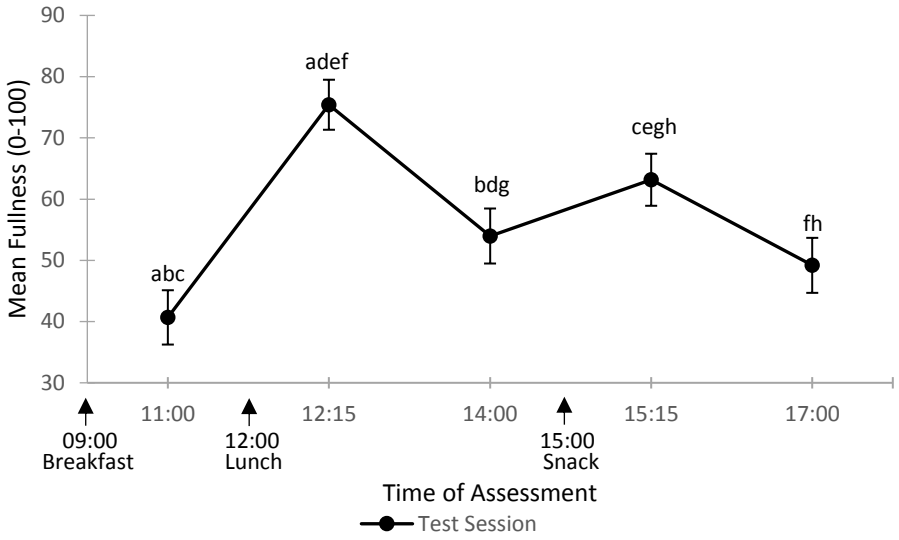


Figure 4.10: Mean Subjective Fullness for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Black arrows represent meal consumption times. Error bars represent standard error of the mean. Baseline fullness (09:15) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure shows that subjective fullness changed throughout the day, with meal consumption having an expected satiating effect.

4.3.4.3 Sleepiness

The overall mean Subjective Sleepiness levels in the FGP condition (M=48.63, SD=13.09) and UGP condition (M=50.95, SD=13.09) did not significantly differ, as indicated by Condition being non-significant. Subjective Sleepiness significantly increased post meal consumption (relative to a pre-meal state) as indicated by post hoc tests following Time being a significant predictor, $F(4, 48) = 10.4$, $p < 0.001$. As shown in Figure 4.11, the highest levels of Subjective Sleepiness were reported two hours after lunch consumption, indicating the presence of a post-lunch dip. The lowest levels of Subjective Sleepiness occurred 15 minutes after snack consumption, suggesting that the snack alleviated the post-lunch dip.

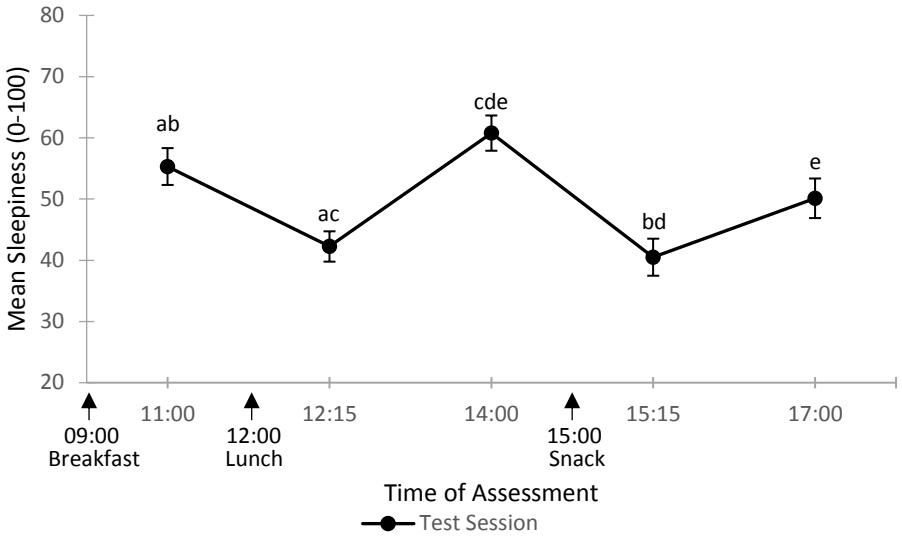


Figure 4.11: Mean Subjective Sleepiness for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Black arrows represent meal consumption times. Error bars represent standard error of the mean. The figure indicates that subjective sleepiness changed throughout the day, with immediate reductions being observed after meal consumption.

The interaction between Time and Regulator Type was a significant predictor of Subjective Sleepiness, $F(4, 48) = 3.768$, $p = 0.01$. As shown in Figure 4.12, post hoc tests revealed that good glucose regulators felt less sleepy immediately after meal consumption, compared to an hour before each meal. Whereas, poor glucose regulators reported more consistent sleepiness levels throughout testing. Good and poor glucose regulators did not significantly differ on overall Subjective Sleepiness levels at any time point. The highest levels of Subjective Sleepiness for both groups were reported two hours after lunch, further indicating the presence of a post lunch dip.

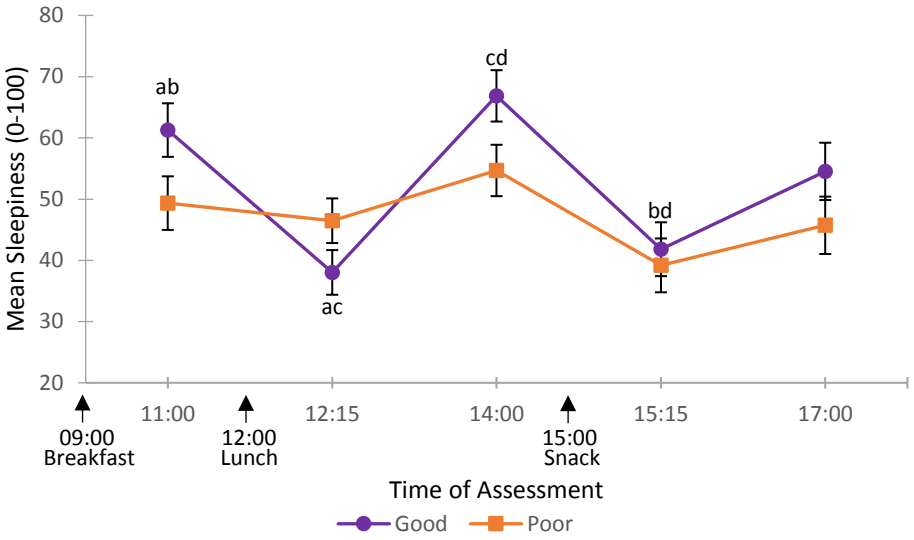


Figure 4.12: Mean Subjective Sleepiness for glucose regulators at each testing session

Values represent estimated marginal means for good (above) and poor glucose regulators (below) at each testing session across conditions. There were 12 good and 12 poor glucose regulators. Test sessions that share the same letter are significantly different from one another for good glucose regulators only. Poor glucose regulators did not significantly differ between sessions. Black arrows represent meal consumption times. Error bars represent standard error of the mean. The figure shows that the good glucose regulators reported significantly different subjective sleepiness levels throughout the day, whereas poor glucose regulators did not.

4.3.5 Summary of findings

The collective findings from the analysis of glycaemic, cognitive performance and subjective mood measures can be found in Table 4.2.

Table 4.4: Summary of glycaemic and subjective mood findings.

Measure	Benefits for FGP	Benefits for UGP	Benefits for good glucose regulation	Benefits for poor glucose regulation
Glycaemic Response	A lower glycaemic response after every meal.	None observed.	A lower glycaemic response after every meal. A lower glycaemic response average in both conditions.	None observed.
Subjective Mood	None observed.	Poor regulators were more content on average compared to FGP.	Lower overall levels of subjective anxiety.	None observed.
Subjective ratings of Hunger, Fullness and Sleepiness	None observed.	None observed.	Lower overall levels of subjective hunger. Lower levels of subjective hunger following UGP lunch and snack.	None observed.

4.4 Discussion

The primary aim of this study was to investigate whether two novel meal profiles produce significantly different glycaemic responses over the course of a testing day. A second aim was to investigate whether any significant differences in subjective mood ratings were found between the two glycaemic response conditions. The final aim was to investigate whether glucose tolerance status within the sample predicted subjective mood ratings.

4.4.1 The Glycaemic Response

The meal manipulations were a success in the sense that the UGP condition produced a significantly higher glycaemic response compared to the FGP condition. This finding is expected as the UGP condition consisted of three HGI meals, which have frequently been demonstrated to produce higher glycaemic responses compared to LGI meals (Brand et al., 1985; Jenkins et al., 1981, 2002; Wolever et al., 1991; Wolever, 2006). The most notable differences occurred post lunch consumption with the UGP condition producing a significantly higher glycaemic response, followed by a sharp drop in glucose concentration, resulting in a short postprandial dip before the afternoon snack. In contrast, a lower glycaemic response was produced by the FGP condition post lunch, which was followed by relatively stable glucose concentrations being maintained until snack consumption. Considering the components of the two lunch meals, it is likely that the differences in fibre content are affecting the glycaemic response produced. According to the dietary fibre hypothesis (Burkitt & Trowell, 1977) a food product that contains very little fibre is more quickly absorbed by the gut compared to a high fibre food. The GI concept (Jenkins et al., 1981) tells us that a quicker absorption rate would then present itself as a more rapid initial increase in glucose concentration, as seen in the UGP condition post lunch. The peak value and rate of glucose concentration decline following the initial rise can be more readily explained by the insulin secretion and sensitivity of participants. During periods of raised glucose concentration, the pancreatic beta cells will secrete insulin that binds to glucose transporter type 4 (GLUT4) proteins on the cellular surface of adipose tissues and skeletal muscles, facilitating the diffusion of circulating glucose down the concentration gradient into muscle and fat cells (James et al., 1988; Kahn & Pessin, 2002; Watson et al., 2004). The ability of cells to respond to insulin is known as insulin sensitivity, with greater insulin resistance being found in those with a poorer glucose tolerance status such as T2DM (ADA, 2014; Wang, 2014). Previous research has found that a greater insulin response can be expected to occur after a greater glycaemic response in healthy individuals (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997). Larger and more rapid insulin responses can result in an insulin spike (i.e. temporary hyperinsulinemia), where too much insulin has been quickly secreted by the pancreas in response to hyperglycaemia (Crofts, 2015). In severe cases, fast rates of insulin-regulated glucose diffusion of the cells can result in reactive hypoglycaemia where blood glucose concentrations reach abnormally low levels (Cryer, Fisher & Shamoon, 1994). Given that the present sample all had normal glucose tolerance, it is likely that the rapid hyperglycaemia elicited by the UGP lunch led to a larger secretion of insulin compared to the FGP lunch, causing an insulin spike. This would result in a quick glucose uptake by muscle and fat cells, presenting itself as a rapid drop in blood glucose

concentration and a postprandial dip as seen in the UGP condition post lunch consumption. However, it is important to note that insulin was not measured in the present study and thus this theory is not confirmed by the current data. Although not immediately obvious due to their relative starting positions pre-snack, the UGP snack also led to a greater glycaemic response compared to the FGP condition. Similar to the glycaemic response post lunch, this finding can be attributed to the lower fibre content of the UGP snack leading to quicker gut absorption rates and thus a greater glycaemic response (Burkitt & Trowell, 1977; Jenkins et al., 2006).

Interestingly, the glycaemic response profile for the FGP and UGP conditions following breakfast consumption is similar with a slight delay appearing in the UGP condition (i.e. the pattern is the same but occurs later for the UGP). The mean glycaemic response is higher for the UGP condition, as might be expected from consumption of a HGI meal (Wolever et al., 1991; Wolever, 2006). Considering the components of the two breakfast meals, it is plausible that the apple juice that was consumed in the FGP condition affected the glycaemic response produced. As aforementioned, a liquid with very little fibre would be quickly absorbed by the gut and result in a rapid initial increase of glucose concentration, which is seen in the FGP condition post breakfast (Burkitt & Trowell, 1977; Jenkins et al., 1981). This rapid increase in glucose concentration could lead to swift insulin secretion of the pancreas which would explain the rapid reduction of glucose concentration at the end of the FGP glycaemic response (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997; James et al., 1988; Kahn & Pessin, 2002; Watson et al., 2004). The role of insulin may also explain the slower decline of glucose concentration in the UGP condition, with a delayed glycaemic response leading to a slower release of insulin, thus a slower glucose uptake by cellular tissues. With the rapid absorption rate of apple juice by the gut, it is also possible that the fructose content and its effect on glucokinase activity plays a role in the sharp drop in glucose concentration shown in the FGP glycaemic response to breakfast. Glucokinase is an enzyme that phosphorylates glucose into glucose-6-phosphate, as well as regulates the glucose uptake rate of the liver and influences rates of glucose storage and glucose output of the liver. Previous research has found that fructose may increase glucokinase activity, which could lead to increased hepatic glucose uptake and reduced hepatic glucose output (Le & Tappy, 2006; Wolever et al., 2009; Wolf et al., 2002). The result of this would be the reducing of postprandial glycaemia, which may contribute to the sharp decrease in glucose concentration shown in the FGP condition post breakfast. Given the crossover nature of the study, increased glucokinase activity due to fructose would be a viable explanation for the quicker decline in glucose concentration seen in the FGP condition post breakfast. Although, it is important to note that the previous evening meal was not standardised for this study, meaning that a potential second meal effect could be present. Previous research has found consumption of an LGI evening meal to be associated with a lower glycaemic response at breakfast compared with an HGI evening meal (Axelsen et al., 1999; Nilsson et al., 2006, 2008; Stevenson et al., 2005; Wolever at al., 1988). In the present instance, participants who consumed a HGI evening meal before consuming the FGP breakfast could produce a higher glycaemic response. It would also be possible that participants who consumed a LGI evening meal before the UGP breakfast produced a lower glycaemic response than they would have if the evening meal was standardised. Therefore, interpretation of the glycaemic response to the breakfast meals should consider this potential

confounding second meal effect. Overall, glucose concentration for both conditions post breakfast returned to baseline within 120-180 minutes, which is indicative of normal glucose tolerance and good insulin sensitivity (Sadler, 2011).

Regarding glucose tolerance status, the results were as expected with poor glucose regulators producing significantly higher glycaemic responses to all test meals across the day compared to good glucose regulators. According to current glucose tolerance diagnostic criteria (WHO, 1999, 2006) the present sample were all of normal glucose tolerance with fasting glucose levels under 6.1mmol/L, which may suggest differences between good and poor regulators would be inconsequential in this range. However, given the crossover study design and the energy and macronutrient matching of the meals, the findings demonstrate clear differences in glycaemic response in relation to glucose tolerance status in a healthy sample. Indeed, the shift from one categorisation of glucose tolerance status to another is not instant and the deterioration of glucose tolerance is the result of a cascade of harmful processes such repeated oxidative stress and inflammation promoting cellular damage and diabetic development and progression (Foirentino et al., 2013; Sharma et al., 2010; Ullah et al., 2015). Given this continuum of glucose tolerance, the comparison of good and poor regulators within a healthy sample warrants examination as well as cross-category comparisons. It is important to note that the classification of good and poor glucose regulators in this study was based on a composite score of eight variables, which were derived from the glycaemic response data (see Chapter 3 for calculation method). Therefore, the results when comparing good and poor glucose regulators are as expected due to the origin of the glucose composite score parameters. The use of a composite score has been argued to have greater ecological validity rather than a single glucose tolerance parameter, which may only account for one part of the glycaemic response (Lamport et al., 2009).

4.4.2 Subjective Mood

The subjective mood findings indicated that no significant differences were found between conditions for all three mood factors; alertness, anxiety and contentment, which suggests that an acute manipulation of glycaemic response through GI variation has minimal impact on subjective mood. A possible explanation for this is that the present sample were all healthy adults with a normal glucose tolerance status, which suggests they have not experienced the many hyperglycaemic episodes required to affect mood. The findings also showed that overall levels of anxiety and contentment did not significantly differ throughout the day. This may reflect the consistent testing environment that participants experienced throughout both experimental conditions. Generally, subjective alertness increased after each meal consumption whilst declining in between meals. The lowest levels of subjective alertness were reported at 14:00, indicative of a post lunch dip in performance. Previous research has reported the presence of a post-lunch dip, often occurring in the mid-afternoon hours, due to the natural circadian rhythm of human beings (Monk, 2005). The severity of this dip has been shown to be increased by consuming a high-CHO lunch, but can still occur even with a light lunch or meal omission (Colquhoun, 1971; Craig et al., 1981). The large rise in alertness levels between 14:00 and 15:15, suggests that this post-lunch dip is alleviated through the

consumption of the snack. Subjective alertness was also found to significantly increase 15 minutes post consumption of each meal, and significantly decrease 95 minutes later. These findings suggest that alertness levels are closely linked to general meal consumption as well as the time of day an assessment is made.

Interestingly, the results indicated that poor glucose regulators reported significantly higher levels of overall anxiety compared to good glucose regulators. A possible mechanism for this may be hyperactivity of the hypothalamic pituitary-adrenal (HPA) axis, which would result in increased levels of glucocorticoids such as cortisol (Reagan et al., 2008). Abnormalities in glucose tolerance have been associated with HPS axis dysfunction, although the underlying mechanisms are unknown (Chan et al., 2005; Reagan et al., 2008). Research has found that higher levels of circulating cortisol to be associated with negative mood outcomes and increased risk of affective disorders such as bipolar disorder (Handley et al., 1980; Van Eck et al., 1996; Ellenbogen et al., 2004, 2010). Finally, poor glucose regulators reported being more content during the UGP relative to the FGP. Interestingly, this finding is similar to that of Pais et al. (2007) who found that T2DM sufferers rated higher on the well-being scale and felt less anger during acute hyperglycaemia compared to an euglycaemic state. Research into T2DM sufferers has found rates of depressed mood to decline after insulin initiation, suggesting insulin may have a positive effect on mood (Ascher-Svanum et al., 2015). As the poor glucose regulators are experiencing higher glycaemic responses in the UGP condition, it is likely that higher insulin responses are also occurring (Holt, Brand-Miller & Petocz, 1997; Seltzer et al., 1967). Therefore, the poor glucose regulators may be reporting higher levels of contentment in the UGP condition as a result of increased insulin levels.

4.4.3 Subjective ratings of Hunger, Fullness and Sleepiness

Results indicated that poor glucose regulators remained significantly hungrier after eating in the UGP condition compared to good glucose regulators. Given that both glucoregulatory groups consumed the same meals throughout the UGP condition, this finding suggests that glucose tolerance plays a moderating role in the relationship between the glycaemic response and subjective hunger. Although all participants in the current sample were of normal glucose tolerance status, it is plausible that the poor glucose regulators have lower insulin sensitivity (i.e. greater insulin resistance) than the good glucose regulators. An increase in insulin resistance would result in a slower rate of glucose uptake by cellular tissue such as muscle and fat cells (James et al., 1988; Kahn & Pessin, 2002; Watson et al., 2004). A potential sign of increased insulin resistance would be extended periods of hyperglycaemia in poor glucose regulators compared to good glucose regulators when consuming the same meals, as reported in the present study (see section 4.3.2). Interestingly, previous research has reported a relationship between subjective hunger and hyperglycaemia, with polyphagia being associated with a hyperglycaemic state (Srinivasan & Ramarao, 2007; Triplitt, 2012; Nakamura, 1962). Furthermore, a positive relationship between hunger and insulin levels has also been indicated, with increased hunger being associated with hyperinsulinaemia (Rodin et al., 1985). Considering greater glycaemic responses were produced in the UGP condition, a greater insulin response would be expected (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller &

Petocz, 1997). Larger and more rapid insulin responses can result in an insulin spike (i.e. temporary hyperinsulinaemia), where too much insulin has been quickly secreted by the pancreas in response to hyperglycaemia (Crofts, 2015). It is plausible that a hyper-insulinaemic episode may last longer in a poor glucose regulator due to increased insulin resistance of cellular tissue (ADA, 2016). Taken together, these findings suggest that the poor glucose regulators are reporting significantly higher levels of hunger after each UGP meal, compared to the good glucose regulators, due to longer periods of hyperglycaemia and possibly hyperinsulinaemia, which have been previously associated with increased hunger (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997; Srinivasan & Ramarao, 2007; Triplitt, 2012; Nakamura, 1962).

Findings also indicated that good glucose regulators reported significantly different levels of subjective sleepiness throughout the day, whereas poor glucose regulators did not. The consistent levels of subjective sleepiness reported by poor glucose regulators across the day may be explained by elevated ghrelin levels. Ghrelin is a peptide hormone that stimulates hypothalamic brain cells to increase hunger and is produced by ghrelinergic cells in the gastrointestinal tract (Dickson et al., 2011; Inui et al., 2004; Meier & Gressner, 2004; Sakata & Sakai, 2010; Schwartz et al., 2000). Ghrelin is secreted when the stomach is empty, but secretion stops when the stomach is stretched through food consumption. Research has found an inverse relationship between ghrelin and insulin with circulating ghrelin levels decreasing in response to insulin administration (Broglio et al., 2004; Chabot et al., 2014). Given that insulin secretion is negatively affected as poorer glucose tolerance develops (ADA, 2014), it is possible that the poor glucose regulators within the sample are secreting less insulin compared to the good glucose regulators after meal consumption. Previous research has found an increase in ghrelin levels to be associated with lower sleepiness and shorter sleep duration (Taheri et al., 2004). Thus, poor glucose regulators may be reporting more consistent levels of subjective sleepiness than good glucose regulators due to comparatively higher levels of ghrelin stimulating hunger and depressing sleepiness.

4.5 Conclusions

This study demonstrates that the two novel meal profiles do produce significantly different glycaemic responses throughout the day in a healthy sample. Specifically, the UGP condition was associated with larger glycaemic responses compared with the FGP condition, with the largest differences occurring post lunch consumption. This finding supports the application of GI to mixed meals in order to manipulate the glycaemic response (Wolever et al., 1985). Good glucose regulators produced significantly lower glycaemic responses than poor glucose regulators demonstrating the use of a glucose composite score is an appropriate defining tool within a healthy sample. Largely, there was little evidence to support that the different meals were associated with clear differences in subjective mood outcomes. However, there was some evidence that poorer glucose regulators showed higher anxiety levels and were less likely to feel full following the meals, indicating glucose tolerance status may affect subjective mood outcomes, although this needs further confirmation. Overall, the principle aim was achieved: to design two multi-meal conditions which show clear differences in glycaemic

response in healthy adults. The next question of interest is whether these glycaemic profiles have measurable effects on cognitive function.

Chapter 5

Utilising GI: Exploring an Optimum Glycaemic Profile for Cognitive Function Across the Day (Study 2)

5.1 Introduction

Despite its' high demand for glucose, the brain has been shown to possess very little glycogen stores, meaning that glucose must be acquired from the blood via the blood brain barrier (Amiel, 1994; Gomez-Pinilla, 2008; Weiss, 1986). Literature investigating the link between glucose availability and cognitive function has demonstrated that glucose ingestion enhances cognitive performance compared to placebo or meal omission conditions (Defeyter & Russo, 2013; Kaplan et al, 2000; King et al, 1945; Korol & Gold, 1998; Smith et al., 1994; Messier et al., 1999). Previous research has suggested a positive relationship between the cognitive demand of a task and the glucose demands of the brain (Donohoe and Benton, 1999; McNay et al. 2000; Reivich and Alavi, 1983; Scholey et al. 2006). Considering this positive association, the rate of glucose release is an important factor in the availability of glucose for the brain, which may have cognitive implications. GI has been shown to reliably predict the rate of glucose release following consumption of single foods and mixed meals, which is reflected in the postprandial glycaemic response (Jenkins et al., 1981; Wolever & Jenkins, 1986; Wolever, 2006). This has made GI a useful vehicle to investigate the relationship between glycaemic response and cognition.

The majority of research that utilises GI to investigate the link between glycaemic response and cognitive performance has used a single meal testing paradigm, largely focussed on the breakfast meal (Boyle et al., 2018). The findings from these studies have been mixed with cognitive benefits being reported following either LGI or HGI meal consumption, whilst some studies have found no significant differences between conditions (Philippou & Constantinou, 2014). As described in Chapter 2, the current evidence is in favour of LGI meal consumption for improved cognitive performance, particularly in areas of memory and attention (Blaak et al., 2012; Philippou & Constantinou, 2014). Previous reviews of the current literature have highlighted methodological limitations such as failure to match meals for energy and macronutrient content, making interpretation of glycaemic and cognitive differences difficult (Adolphus et al., 2016, Philippou & Constantinou, 2014). Given that the theory explored in these studies is that cognitive performance is linked to glycaemic response, one approach is to ensure conditions produce clearly different glycaemic response profiles before cognitive testing takes place. Once measurably different glycaemic response profiles have been established between conditions, assessment times of cognitive performance can be appropriately selected to test this theory. Thus, two novel meal profiles were assessed in study 1 of this thesis to investigate whether they produced significantly different glycaemic response profiles across the day. The previously used single meal testing paradigm was extended to the investigation of three consecutive mixed meals to reflect everyday conditions. Meals were isoenergetic and macronutrient matched to prevent confounding factors on glycaemic response. As expected, Chapter 4 showed that the FGP condition produced a significantly lower glycaemic response profile compared to the UGP condition. Having

established that two different glycaemic responses can be expected from the consumption of the meal profiles, it was then appropriate to assess cognitive performance during each condition. Therefore, the primary aim of this study was to investigate whether there were any significant differences in cognitive performance between the two glycaemic response conditions. To address this aim, a cognitive task battery was implemented multiple times throughout the day in both the FGP and UGP conditions. Assessment times were the same across conditions to allow comparisons at each testing session.

The glucose tolerance status of an individual has been previously shown to be associated with cognition. Specifically, poorer glucose tolerance has been associated with increasing cognitive impairment (Awad et al., 2004; Cukierman et al., 2005; Geijselaers et al., 2015; Messier, 2005). It is also speculated that the cognitive deficits seen with poorer glucose tolerance increase with age, and that individuals with poorer glucose tolerance benefit more from glucose consumption compared to healthy individuals (Lamport et al., 2009). Previous research also indicates that the continued consumption of a LGI diet can improve glucose tolerance measures such as HbA1c levels, which may attenuate some cognitive impairment (Thomas & Elliot, 2009). Considering the link between glucose tolerance and cognition, the relative impact of glucose tolerance status on the cognitive effects on the two meal profiles warrants investigation. For example, previous research has shown that LGI foods (relative to high GI) are particularly beneficial for participants with poor glucose tolerance (Papanikolaou et al., 2006). Therefore, the second aim of this study was to investigate whether glucose tolerance status within a healthy sample predicts cognitive performance. To address this aim, the sample were split into good and poor glucose regulators using a glucose composite score (see Chapter 3).

Similar to study 1, potential mood differences between glycaemic response conditions and glucose tolerance statuses were also considered. Individuals with poor glucose tolerance have often been shown to display worsened mood and higher depression rates compared to good glucose regulators (Maraldi et al., 2007; Nabb & Benton, 2006; Penckofer et al., 2012). Furthermore, long term improvements in glucose tolerance have been associated with an improvement in mood (Lustman et al., 2007). In acute testing conditions, there is consistent evidence that general meal consumption is associated with improved mood compared to meal omission (Adolphus et al., 2016). However, the acute comparison of mood effects between GI conditions has returned mixed results, with some studies reporting benefits from either a LGI or HGI meal, whilst others found no significant differences (see Chapter 2). Therefore, the third aim of this study was to investigate whether there are any significant differences in subjective mood between two glycaemic response conditions. Furthermore, to consider glucose tolerance, the fourth aim of this study was to investigate whether glucose tolerance status within a healthy sample predicts subjective mood outcomes.

5.1.1 Summary of aims

Aim 1: To investigate whether there are any significant differences in cognitive performance between a favourable glucose profile (FGP) and an unfavourable glucose profile (UGP) in a healthy population.

Aim 2: To investigate whether glucose tolerance status within a healthy population predicts cognitive performance.

Aim 3: To investigate whether any significant differences in subjective mood ratings are found between the FGP and UGP within a healthy population.

Aim 4: To investigate whether glucose tolerance status within a healthy population predicts subjective mood ratings.

5.1.2 Study Hypotheses

Hypothesis 1: The FGP condition will be associated with improved cognitive performance across the day compared to the UGP condition.

Hypothesis 2: Good glucose regulators will display significantly better cognitive performance across the day compared to poor glucose regulators within the sample.

Hypothesis 3: The FGP condition will be associated with improved subjective mood ratings across the day compared to the UGP condition.

Hypothesis 4: Good glucose regulators will display significantly better subjective mood across the day compared to poor glucose regulators within the sample.

5.2 Method

5.2.1 Power analysis

A prior power analysis was conducted using Gpower 3.1 to determine the sample size required. Assuming an effect size of d = 0.59 (Mahoney et al., 2005 experiments 1 & 2) with a statistical power of 0.95 and an alpha level of 0.05, 40 participants were deemed sufficient to detect cognitive performance differences between conditions. Extra participants were recruited in the event of a drop out to ensure minimum sample size requirements were achieved.

5.2.2 Recruitment

Opportunistic sampling involved sending emails to relevant group mailing lists, the handing out of fliers and posters, as well as posting on the University of Reading campus and Reading town notice boards. All participants were recruited from the local population within the county of Berkshire.

5.2.3 Participants

Forty healthy, normal weight adults (20 males and 20 females), with a mean age of 21.43 years (SD = 0.32) (see section 5.3.1 for full details). Inclusion criteria were aged between 18–25 years old and a BMI of 18.5-25kg/m^2. All participants were self-reported healthy non-smokers, with no relevant food intolerances or allergies.

Exclusion criteria were a medical diagnosis of high blood cholesterol or pressure, any condition that could affect glucose metabolism (e.g. diabetes, anaemia and pregnancy), or a disease of complication which affected their thyroid, heart, brain, vascular system, bones, kidneys, gastrointestinal tract, respiratory system or liver. Prescribed anti-depressants was also an exclusion criterion as these drugs have the potential to impact glucose metabolism (Deuschle, 2013; Himmerich, Minkwitz & Kirkby, 2015) and cognitive function (Hindmarch, Kimber & Cockle, 2000; Knegtering, Eijck & Huijsman, 1994). Finally, any participant that was a self-reported professional athlete was excluded, as these individuals could have improved glucose uptake due to skeletal muscular adaptations such as increased GLUT4 glucose transporter expression (Goodyear & Kahn, 1998). All exclusion criteria were checked through self-reporting on the health & lifestyle medical questionnaire, which participants completed and returned by email before a screening session could be arranged (Appendix H).

5.2.4 Study design

The present study followed a counterbalanced, randomised, crossover design using the same two experimental conditions implemented in Study 1. The order in which a participant took part in the experimental conditions was obtained by entering participant numbers into an online researcher randomiser (Urbaniak & Plous, 2013). To allow for potential dropouts, twice the number of required participants was entered into the randomiser prior to the study. This resulted in half of the sample beginning with the FGP condition, whilst the other half took part in the UGP condition initially. For the order of participation see Appendix E. The two conditions were (i) Favourable Glycaemic Profile (FGP) - a LGI diet consisting of a breakfast, lunch and afternoon snack and (ii) Unfavourable Glycaemic Profile (UGP) - a HGI diet consisting of a breakfast, lunch and afternoon snack. The independent variables were Condition, Time and Regulator Type, whilst the dependent variables were Cognitive Performance, Glycaemic Response, Subjective Mood, Hunger, Fullness and Sleepiness ratings.

5.2.5 Nutritional manipulations

The conditions were identical to those described in Chapter 4. All macronutrient compositions along with GI/GL values can be found in sections 4.2.5.1 and 4.2.5.2.

5.2.6 Outcome variables

5.2.6.1 Glycaemic Response

Interstitial glucose was measured using the continuous glucose monitoring procedure detailed in section 3.5.2. During each testing day, a total of twenty-three interstitial glucose readings were taken. The assessment times for the readings were; immediately before each meal (0 minutes), and 15, 30, 45, 60, 90, 120, and 150 minutes post meal consumption (See 5.1). This procedure allowed glucose measurements to be taken regularly without interrupting meal consumption or cognitive performance and subjective mood assessments (See 5.1 for test day procedure).

5.2.6.2 Cognitive Performance

Cognitive performance was assessed using the cognitive task battery described in Chapter 3. Each task consisted of two outcome measures; Accuracy and Reaction Time (for correct responses only). The outcome variables of Global Cognitive Accuracy and Global Cognitive Reaction Time were calculated retrospectively using the Accuracy and Reaction Time measures from all tasks (see Chapter 3). To allow sufficient completion time of the cognitive battery and to avoid delaying meal consumption, cognitive assessments occurred twenty minutes prior to each meal, as well as thirty- and ninety-minutes post meal serving. Including the baseline reading taken at the start of a test day, cognitive performance was assessed a total of nine times during each experimental condition.

5.2.6.3 Self-report measures (questionnaires)

A total of 6 subjective mood, hunger, fullness and sleepiness self-evaluations were carried out throughout each test day. Subjective mood was measured using Bond & Lader Mood VAS (Bond & Lader, 1974) (see section 3.7.1). The outcome variables for this were scores of 0-100 for alertness, anxiety and contentment mood factors. Similarly; hunger, fullness and sleepiness were also measured using VAS (see section 3.7.2). The outcome variables for this were scores of 0-100 for each of the three factors. Full details of these can be found in Chapter 3.

5.2.7 Procedure

5.2.7.1 Screening

Potential participants who contacted the researcher were sent by email the health and lifestyle medical questionnaire (Appendix H) which they were required to return before a screening session was arranged. The questions contained within this questionnaire ensured that the participant met inclusion and exclusion criteria (see section 5.2.3 for inclusion and exclusion criteria). If a participant's response fulfilled the criteria, a one-hour screening session was arranged for the morning, at the University of Reading's Hugh Sinclair Unit of Human Nutrition. The screening session was similar to study 1 with data relating to height, weight,

blood pressure and fasting glucose levels being collected (see Chapter 4, section 4.2.7 for screening equipment and procedure). The screening form used by the experimenter for each measure can be found in Appendix J. The only difference between study 1 and the present study's screening procedure was the addition of a practise run of the cognitive task battery. This involved the participant carrying out the cognitive battery once in order to familiarise themselves with the cognitive tasks as recommended by Bell et al. (2018). Once screening was completed, the test days were arranged, the first of which was required to be a least one week following the screening session.

5.2.7.2 Testing

5.2.7.2.1 Prior dietary instructions

Participants were asked to refrain from the consumption of alcohol and avoid any form of exercise for the 24 hours prior to a test visit. Participants were provided with a standardised meal to consume in the evening prior to testing. This meal consisted of two slices of plain white Hovis bread and a tin of Heinz Baked Beans. Participants were instructed to consume their evening meal prior to the day of testing between the hours of 6 and 8pm, and to keep this time consistent the evening before each test day. After the evening meal, participants were required to fast (no food or drink except water) for the rest of the day.

5.2.7.2.2 Day sessions

Participants were required to arrive at the Hugh Sinclair Unit of Human Nutrition in a fasted state from waking, having consumed nothing expect water on the morning of a test day. The schedule of a test day is shown in Figure 5.1. Upon arrival at 08:00 participants had a continuous glucose sensor attached to the back of their upper left arm. Whilst the sensor self-calibrated, the participant waited in a quiet room where they were able to watch television or read materials such as magazines provided within the Research Unit. The first cognitive (baseline) assessment began at 08:40 followed by a interstitial glucose reading being taken immediately before the consumption of breakfast at 09:00. Participants were required to consume all of the test meal within 15 minutes. The cognitive battery implemented in this study lasted approximately 20 minutes (see Chapter 3). Cognitive assessments were initiated twenty minutes prior to each meal, then 30- and 90-minutes post meal serving. A total of twenty-three glucose readings were taken throughout the day, with a reading being taken immediately before each meal (0 minutes), and 15, 30, 45, 60, 90, 120- and 150-minutes post meal consumption. Subjective mood, hunger, fullness and sleepiness were self-evaluated at 15- and 105-minutes post meal consumption. The test day ended at 17:00 (see Figure 5.1).

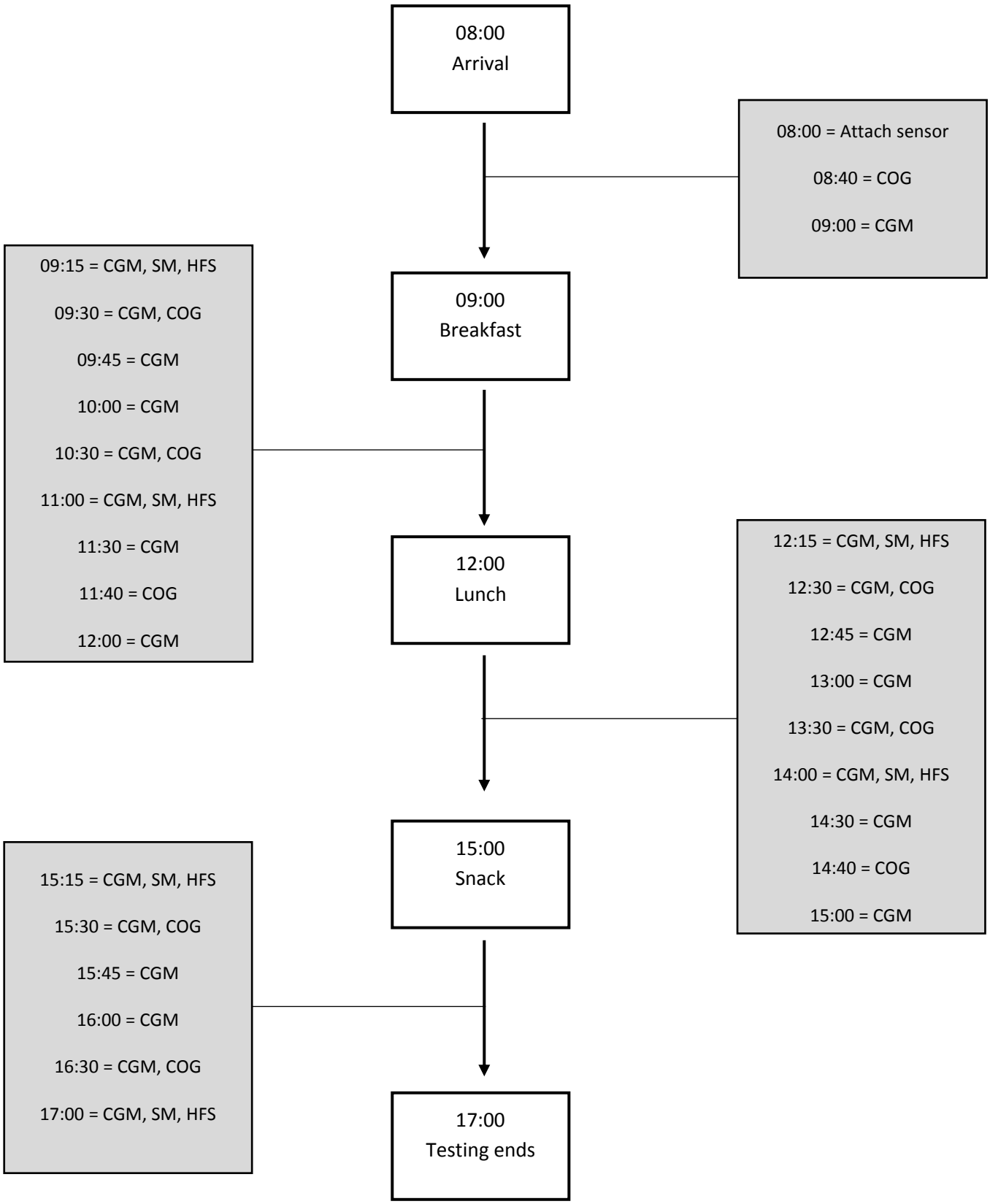


Figure 5.1: A flow diagram showing the step-by-step procedure for each test day

*CGM = Continuous Glucose Monitor reading, COG = Cognitive performance assessment, SM = Subjective Mood evaluation, HFS = Hunger, Fullness, Sleepiness evaluation.

5.2.8 Payment

The participant payment amounts were standardised in line with the Hugh Sinclair Unit of Human Nutrition guidelines. All participants received £50 per visit, giving a total of £100 paid to each. This amount was considered sufficient to financially remunerate participants for their time and any incurred travel expenses.

5.2.9 Ethical approval

This study received a favourable opinion for conduct from the School of Psychology and Clinical Language Sciences Ethics Committee (SREC) and the University of Reading Ethics Committee (UREC). Evidence of ethical approval can be found in Appendix L. The clinical trials ID number for this study is NCT03346746.

5.2.10 Statistical analysis

Linear Mixed Modelling was used to analyse the data, with the specific LMM presented in Chapter 3, Section 3.5.2 being carried out on each outcome variable. Predictors of outcome variables and interactions of independent variables are only fully reported if statistically significant ($p<0.05$) or approaching significance ($p<0.1$). In both cases, pairwise comparisons were consulted and reported. All F values not reported in the results section are included in Appendix F.

Overall, there were two main outcome variables for each cognitive task (i.e. Accuracy and Reaction Time) with the LMM being conducted on each of these outcome variables. Three further cognitive outcome variables were calculated retrospectively using the Accuracy and Reaction Time scores from the four cognitive tasks; Global Cognitive Accuracy, Global Cognitive Reaction Time and Global Cognitive Performance (see Chapter 3 for calculation method). Additionally, accuracy and reaction time on the CRT task was compared to the CRT aspect within the Merged task (correctly indicating which side “x” had appeared, regardless of identifying a number sequence with the “space” bar) to examine any potentials effects of task difficulty. Similarly, performance on the RVIP task was also compared to the Merged task RVIP performance (correctly pressing the “space” bar when a target number sequence appeared, regardless of whether the position of “x” had been correctly identified). Given that the number of targets varied between tasks, Accuracy scores on each task were initially converted into a percentage. Once converted, percentages from both tasks were placed under a single “Percent Correct” outcome variable, and “Task” was entered as another independent variable into the LMM. All Reaction Time data was measured in milliseconds so no conversion was necessary before the LMM was conducted. The process of comparing task performance resulted in an additional four LMMs being carried out (i.e. two for Percent Correct and two for Reaction Time comparisons). When reporting the LMM analysis of these cognitive task comparisons, only the Task predictor and any interactions it shared with Condition, Time or Regulator Type were reported.

The other outcome variables were; Glycaemic Response, Alertness, Anxiety, Contentment, Hunger, Fullness and Sleepiness. The LMM described in Chapter 3, Section 3.5.2 was run on each outcome variable resulting in a total of twenty-two models being performed during the present study analysis. Results deriving from the model are reported below for each outcome variable. For all significant findings, appropriate post hoc tests and bonferroni corrections were carried out and are also reported below.

5.3 Results

5.3.1 Participant characteristics

The characteristics of twenty male and twenty female participants are shown in Table 5.1. All participants were within the 18-25 years age range, as well as the 18.5-25kg/m^2 BMI range.

Table 5.1: Participant Characteristics (n = 40, 20 male, 20 female)

Variable	M	SD	Min	Max
Height (cm)	173.4	1.4	159	191
Weight (kg)	65.7	1.4	48.2	86.8
BMI (kg/m^2)	21.8	0.3	18.5	25
Age (years)	21.4	0.3	18	25
Fasting Glucose (mmol/L)	5.05	0.05	4.2	5.7

Note. Fasting Glucose value was obtained at screening.

*M = Mean, SD = Standard Deviation, Min = Minimum value, Max = Maximum value.

5.3.2 Glycaemic Response

An initial t test indicated that baseline glucose values were significantly higher in the FGP condition (M=3.56mmol/l, SD=0.79) compared to the UGP condition (M=3.36mmol/l, SD=0.91) at the start of the day; $t(39) = 2.067$, $p = 0.045$. As expected, the overall mean glucose concentration was significantly higher in the UGP condition (M=4.47mmol/L, SD=0.56) compared to the FGP condition (M=3.98mmol/L, SD=0.56), as indicated by Condition being a significant predictor $F(1, 142) = 29.388$, $p < 0.001$. The interaction between Condition and Time was also significant for Glycaemic Response, $F(21, 478) = 7.099$, $p < 0.001$. Bonferroni corrected post hoc tests revealed that the glycaemic response was significantly higher in the UGP condition at 45, 60, 90, 120, 150, 195, 210, 225, 240, 390, 405, and 420 minutes (all: $p<0.05$), indicating that longer periods of hyperglycaemia were experienced after the consumption of HGI meals compared to the LGI meals. The glycaemic response was significantly lower in the UGP condition at 360 minutes compared to the FGP condition ($p=0.032$), indicating the presence of a postprandial dip following the HGI lunch (see Figure 5.2).

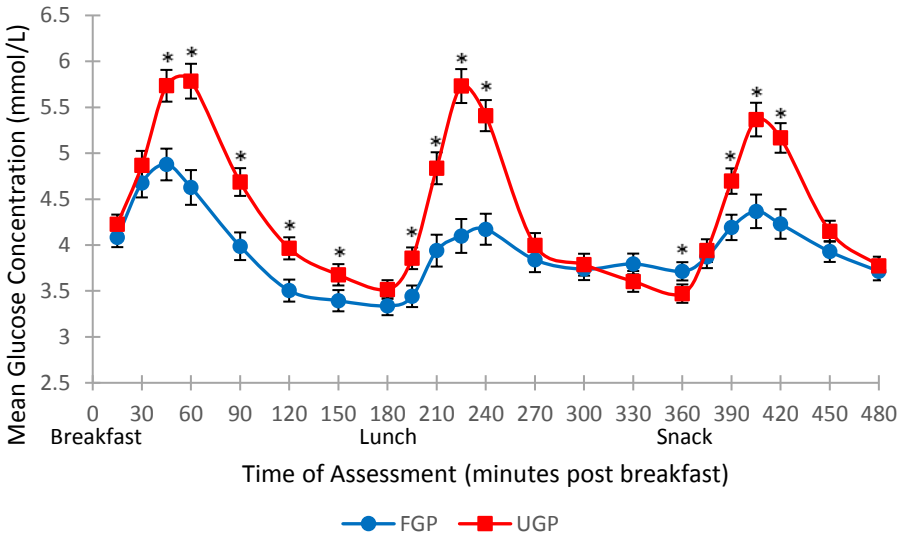


Figure 5.2: The interaction between Condition and Time for Glycaemic Response

Data points represent estimated marginal means across all participants at each time point. Error bars represent standard error of the mean. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Baseline glucose (0 minutes) was included as a covariate in the linear mixed model, hence its exclusion from the figure. Poor glucose regulators produced a significantly higher glycaemic response than good glucose regulators after each meal, apart from immediately before the snack (360 minutes) where glucose concentrations were significantly higher for the good glucoregulatory group

In regards to glucose tolerance status, poor glucose regulators produced a significantly higher glycaemic response average (M=4.59mmol/L, SD=0.61) compared to good glucose regulators (M=3.85mmol/L, SD=0.56), indicated by Regulator Type being a significant predictor, $F(1, 197) = 46.588$, $p < 0.001$. However, neither the three-way interaction between Condition, Time and Regulator Type or the two-way interactions of Regulator Type with Condition or Time were found to predict Glycaemic Response.

5.3.3 Cognitive Performance

5.3.3.1 Choice Reaction Time Task

For raw RT data, there were an initial 42,263 trials with an associated correct response. A total of 68 trials had a RT of under 100ms and were removed. A further 603 trials were identified as potential outliers due to having a Z score of 3.29 or higher and were removed before calculating session means. Histograms demonstrating this outlier process step by step can be found in Appendix M. This task did not contain a false positives element, so all resulting session means were included in Accuracy and RT analyses.

5.3.3.1.1 CRT Accuracy

The overall mean CRT Accuracy scores in the FGP condition (M=58.75, SD=0.82) and UGP condition (M=58.78, SD=0.82) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Accuracy on the CRT task, indicating that the performance of participants did not significantly differ between or within conditions. Regulator Type did not significantly predict CRT task Accuracy either, reflecting similar performance between glucoregulatory groups.

5.3.3.1.2 CRT Reaction Time

The overall mean CRT task Reaction Times in the FGP condition (M=315.74, SD=22.14) and UGP condition (M=316.25, SD=22.14) did not significantly differ, as indicated by Condition being non-significant. The Time predictor was significant for CRT task Reaction Time, $F(7, 80) = 2.225$, $p = 0.041$. Post hoc tests revealed that significantly slower reaction times occurred immediately before snack consumption compared to immediately before lunch consumption, indicating a drop in performance during the mid-afternoon (see Figure 5.3).

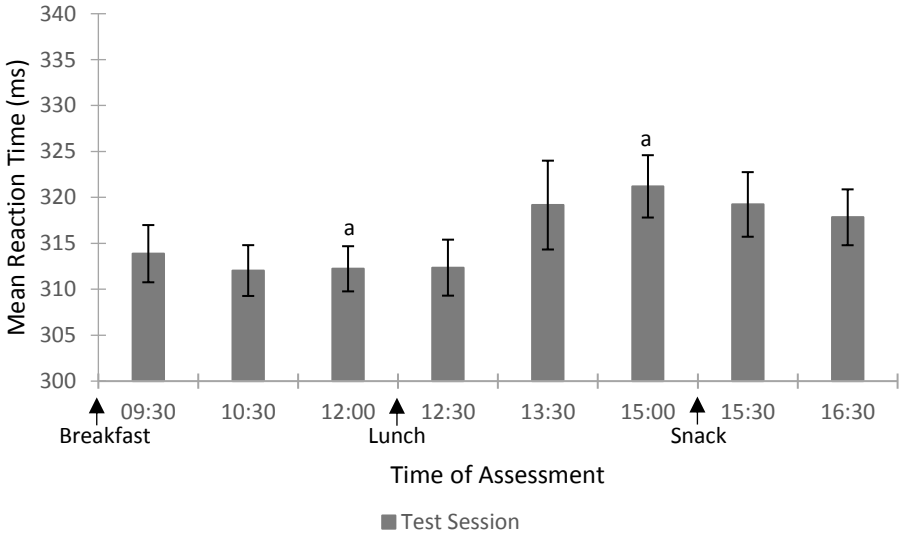


Figure 5.3: Mean CRT task Reaction Time for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure shows that slower reaction times where produced during the afternoon relative to the morning, with significantly slower reaction times occurring at 15:00 compared to 12:00.

The interaction between Condition and Time was also significant for CRT task Reaction Time; $F(7, 80) = 2.633$, $p = 0.017$, and revealed significant differences between assessments within the FGP condition only, with the slowest reaction times occurring at 15:00, also suggesting a

mid-afternoon performance drop (see Figure 5.4). In the FGP condition, significantly faster reaction times were seen at 10:30 compared to 15:00 and 16:30, suggesting that the FGP condition is associated with faster reaction times after breakfast relative to later in the day (see Figure 5.4). No significant differences were seen between glucoregulatory groups as indicated by Regulator Type being non-significant.

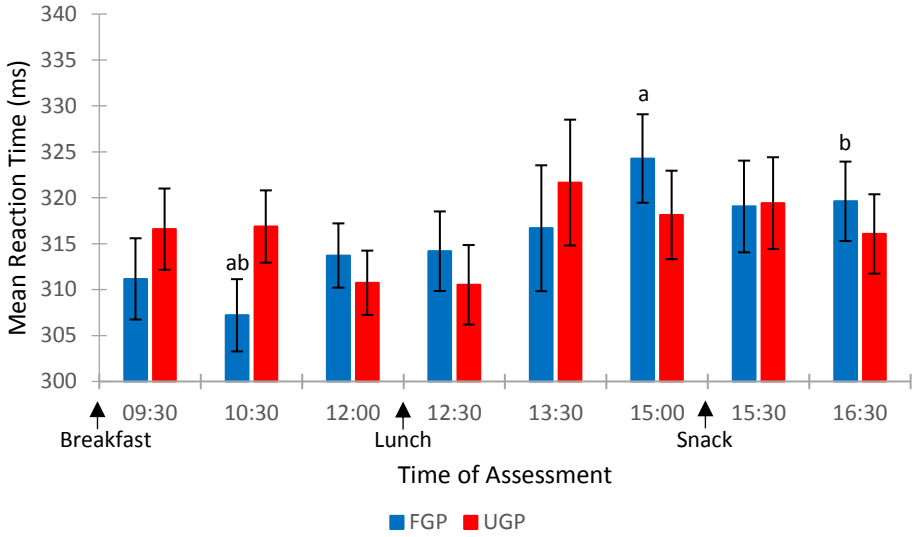


Figure 5.4: The interaction between Condition and Time for CRT task Reaction Time

Values represent estimated marginal means across all participants at each time point. Test sessions that share the same letter are significantly different from one another within condition. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure shows that significantly faster reaction times were recorded at 10:30 compared to 15:00 and 16:30 for the FGP condition. No significant differences were found within the UGP condition. Slowest performance occurred during the mid-afternoon sessions for both conditions.

5.3.3.2 Rapid Visual Information Processing Task

For raw RT data, there were an initial 12,496 trials with an associated correct response. A total of 48 trials had a RT of under 100ms and were removed. A further 148 trials were identified as potential outliers due to having a Z score of 3.29 or higher. However, these 148 trials were not deemed to be sufficient enough outliers after consultation with histograms and consideration of the task's short timeframe (100-800ms), and thus were not removed from subsequent analysis. Histograms demonstrating this outlier process step by step can be found in Appendix M. One participant's data included a baseline session with an associated false positives rate over the 33% cut off, which resulted in this baseline session being removed from both Accuracy and RT analyses.

5.3.3.2.1 RVIP Accuracy

The overall mean RVIP Accuracy scores in the FGP condition (M=17.63, SD=1.81) and UGP condition (M=17.07, SD=1.79) did not significantly differ, as indicated by Condition being non-significant. The Time predictor approached significance for predicting RVIP task Accuracy, F(7, 79) = 1.805, p = 0.098. Although, Bonferroni corrected post hoc tests revealed no significant differences between assessments across conditions. The Condition by Time interaction did not significantly predict RVIP task Accuracy, indicating similar performance throughout both conditions. Regulator Type was not a significant predictor of RVIP task Accuracy, reflecting similar performance between glucoregulatory groups.

5.3.3.2.2 RVIP Reaction Time

The overall mean RVIP task Reaction Times in the FGP condition (M=387.56, SD=31.79) and UGP condition (M=375.48, SD=31.43) did not significantly differ, as indicated by Condition being non-significant. RVIP task Reaction Time significantly varied throughout the day across conditions as indicated by Time being a significant predictor, F(7, 79) = 3.079, p = 0.006. Post hoc tests revealed that significantly slower reaction times were found 30 minutes post lunch compared to immediately before snack consumption, indicating the presence of a post-lunch dip (see Figure 5.5). Interestingly, quicker reaction times were generally quicker in the assessments immediately before meal consumption relative to the assessments post consumption (see Figure 5.5). An example of this can be seen when compared the 12:00 assessments with the 12:30 and 13:30 assessments (see Figure 5.5). Neither Condition or Regulator Type were found to be significant predictors of RVIP task Reaction Time, indicating similar reaction times were produced between conditions and glucoregulatory groups.

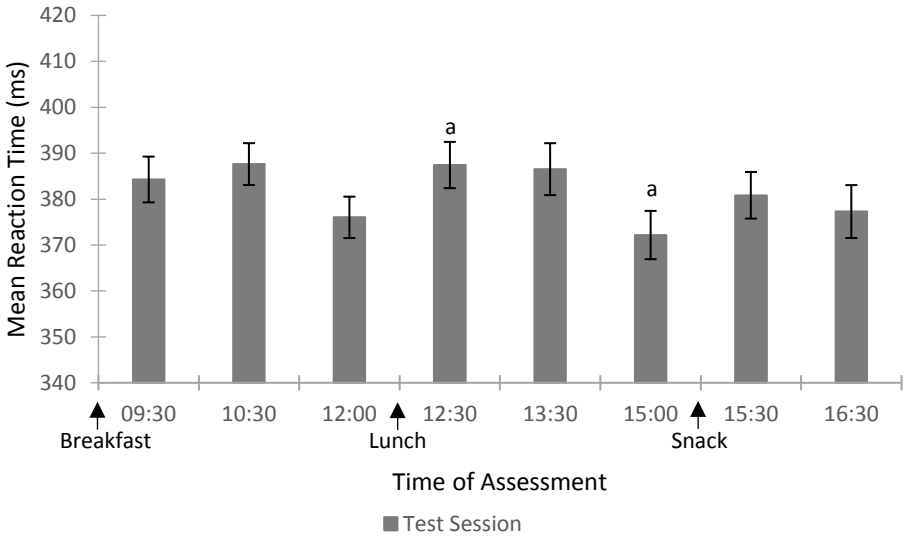


Figure 5.5: Mean RVIP task Reaction Time for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure shows that reaction time remained fairly consistent throughout testing, with the only significant difference between assessments being faster reaction times at 15:00 compared to 12:30, indicative of a post-lunch dip.

5.3.3.3 Merged Task

For raw RT data, there were an initial 9,939 trials with an associated correct response. No trials had a RT of under 100ms, nor did any trial have a Z score of 3.29 or higher. A histogram demonstrating this can be found in Appendix M. One participant's data included a baseline session and a separate testing session with an associated false positive rate over the 33% cut off, which resulted in both sessions being removed from Accuracy and RT analyses.

5.3.3.3.1 Merged Accuracy

The overall mean Merged task Accuracy scores in the FGP condition (M=13.94, SD=3.12) and UGP condition (M=13.68, SD=3.09) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Accuracy on the Merged task, indicating that the performance of participants did not significantly differ between or within conditions. Regulator Type was also non-significant, indicating that similar performance between glucoregulatory groups. However, the two-way interaction between Condition and Regulator Type was found to be a significant predictor of Merged task Accuracy, $F(1, 79) = 4.36$, $p = 0.04$. Differences were limited to the FGP condition where good glucose regulators were significantly more accurate than poor glucose regulators, indicating that the good glucose regulators benefitted from the FGP condition relative to the

poor glucoregulatory group (see Figure 5.6). Neither glucoregulatory group produced significantly different Merged task Accuracy scores between conditions.

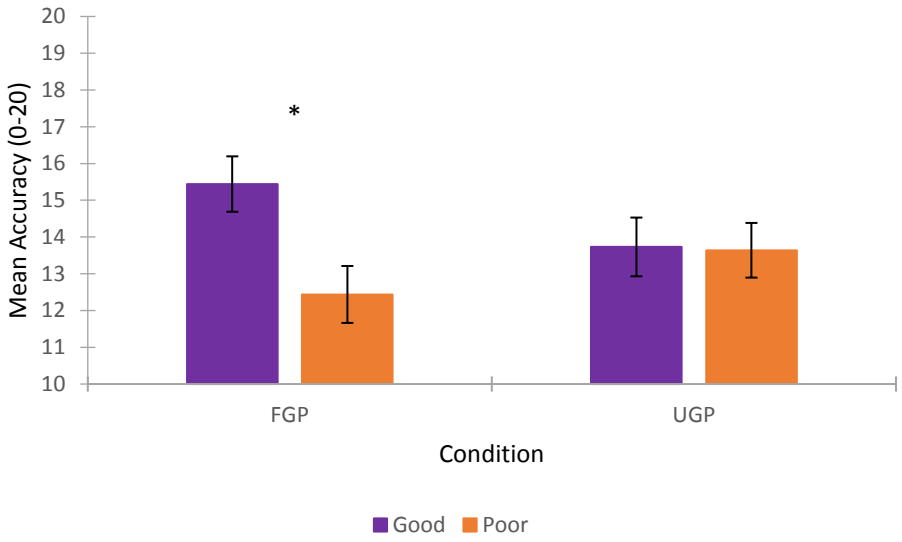


Figure 5.6: Merged task Accuracy condition means for good and poor glucose regulators

Values represent estimated marginal means for good and poor regulators within each condition. There were 20 good and 20 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a condition. Error bars represent standard error of the mean. The figure shows that good glucose regulators were significantly more accurate than poor glucose regulators in the FGP condition.

5.3.3.3.2 Merged Reaction Time

The overall mean Merged task Reaction Times in the FGP condition (M=471.08, SD=86.02) and UGP condition (M=455.25, SD=87.55) did not significantly differ, as indicated by Condition being non-significant. The Time predictor tended towards significance for Merged task Reaction Time, $F(7, 73) = 1.93$, $p = 0.077$. Although, Bonferroni corrected post hoc tests revealed no significant differences between assessments. Neither Condition or Regulator Type were found to be significant predictors of Merged task Reaction Time, indicating similar reaction times were produced between conditions and glucoregulatory groups.

5.3.3.4 Letter Memory Task

For raw RT data, there were an initial 7,767 trials with an associated correct response. No trials had a RT of under 100ms. A total of 76 trials were identified as potential outliers due to having a Z score of 3.29 or higher and were removed before calculating session means. Histograms demonstrating this outlier process step by step can be found in Appendix M. This task did not contain a false positives element, so all resulting session means were included in Accuracy and RT analyses.

5.3.3.4.1 LM Accuracy

Condition approached significance for LM task Accuracy, F(1, 81) = 3.662, p = 0.059, with participants being less accurate in the UGP condition (M=10.38, SD=2.06) compared to the FGP condition (M=11.27, SD=2.06). However, pairwise comparisons indicated that the difference between conditions was not significant. Neither Time or the Condition by Time interaction were found to significantly predict LM task Accuracy, indicating similar performance throughout both conditions. Regulator Type was also not a significant predictor of LM task Accuracy, reflecting similar performance between glucoregulatory groups.

5.3.3.4.2 LM Reaction Time

The overall mean LM task Reaction Times in the FGP condition (M=2337.06, SD=505.03) and UGP condition (M=2273.18, SD=505.03) did not significantly differ, as indicated by Condition being non-significant. LM task Reaction Time significantly varied throughout the day as indicated by Time being a significant predictor, F(7, 80) = 2.189, p = 0.044. Post hoc tests revealed that participants were significantly slower in the morning following breakfast compared to the last afternoon session, with a general improvement throughout the day, suggesting possible practise effects (see Figure 5.7). Neither Condition or the Condition by Time interaction were significant predictors of LM task Reaction Time, indicating similar reaction times were produced across conditions throughout the day.

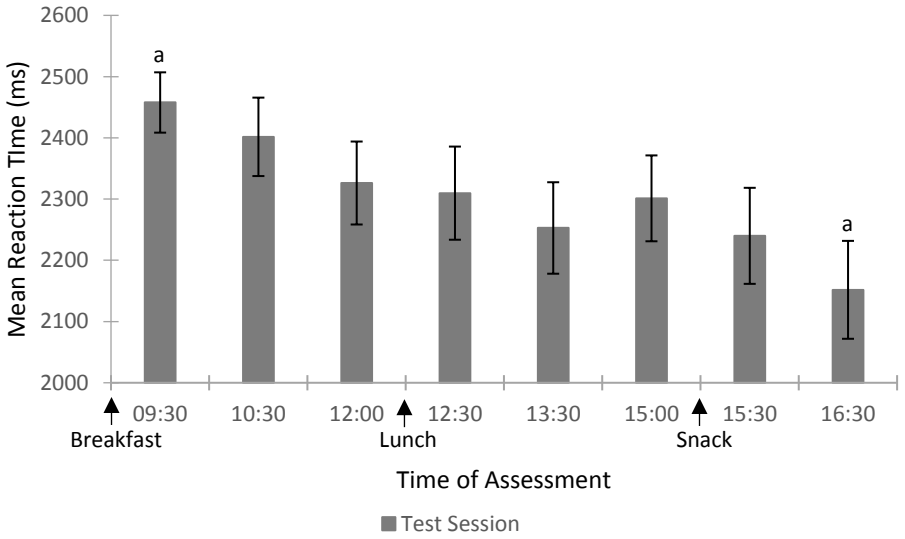


Figure 5.7: Mean LM task reaction time for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure indicates that reaction times became faster throughout the day with the largest difference occurring between the first and last assessments, which suggests the presence of practise effects.

The three-way interaction between Condition, Time and Regulator Type was also significant for LM task Reaction Time, $F(7, 80) = 2.991$, $p = 0.008$. Post hoc tests revealed that good glucose regulators were significantly slower 30 minutes post lunch in the FGP condition compared to the UGP condition, whereas poor glucose regulators did not significantly differ between experimental conditions at any assessment (see Figure 5.8). Interestingly, there appears to be a general pattern in the data whereby good glucose regulators reacted slower, whilst poor glucose regulators reacted quicker, in the FGP condition compared to the UGP condition, which suggests that the FGP meal profile was more beneficial to performance in the presence of poorer glucose tolerance (see Figure 5.8). Post hoc tests also revealed that LM task Reaction Time significantly differed between assessment time points for poor glucose regulators within both conditions (see Figure 5.8). Within the FGP condition, poor glucose regulators were found to be significantly slower 30 minutes post breakfast compared to 30 minutes post lunch, suggesting the LGI lunch had a greater effect than the LGI breakfast. Within the UGP condition, poor glucose regulators were significantly slower 90 minutes after breakfast compared to 90 minutes post lunch. Good glucose regulators did not produce significantly different reaction times between assessments in either condition. No significant differences in mean LM task Reaction Time were found between glucoregulatory groups at any assessment time point within either condition.

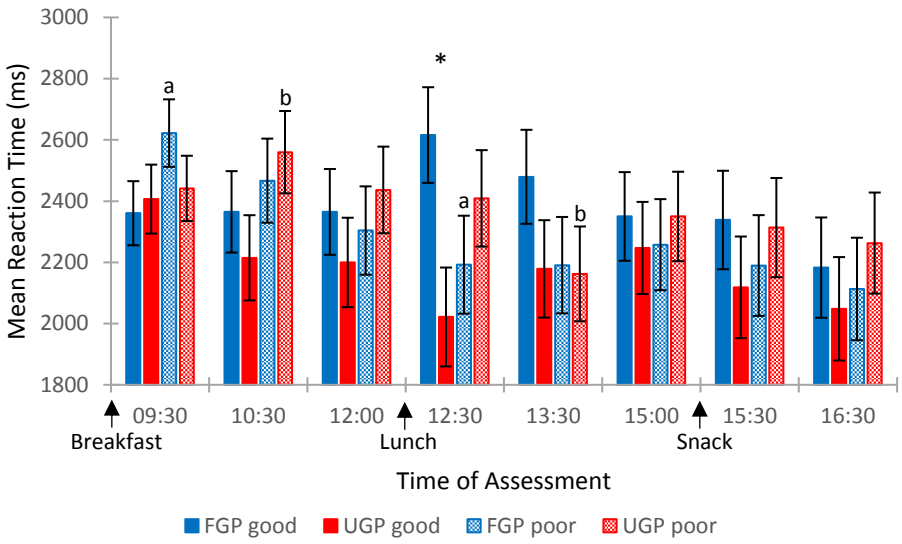


Figure 5.8: Condition means within each glucoregulatory group for LM task Reaction Time

Values represent estimated marginal means for good and poor regulators at assessment time points within each condition (above = good regulators, below = poor regulators). There were 20 good and 20 poor glucose regulators. An asterisk (*) indicates a significant difference between conditions at an assessment time point within the good glucoregulatory group only. Test sessions that share the same letter are significantly different from one another for a glucoregulatory group within condition. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure indicates that good glucose regulators were significantly quicker at 12:30 in the FGP condition compared to the UGP condition, whilst no differences between conditions occurred for the poor glucoregulatory group. The figure also shows that poor glucose regulators were significantly slower in the morning relative to the mid-afternoon in both conditions. No significant differences between assessments were found for good glucose regulators within either condition but reaction times appear to become faster towards the end of the day in both conditions.

5.3.3.5 Global Cognition

5.3.3.5.1 Global Cognitive Accuracy

The overall mean Global Cognitive Accuracy scores in the FGP condition (M=0.084, SD=0.56) and UGP condition (M=-0.111, SD=0.056) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Global Cognitive Accuracy, indicating that the participants did not perform significantly differently between or throughout the FGP and UGP conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups did not display significantly different levels of Global Cognitive Accuracy.

5.3.3.5.2 Global Cognitive Reaction Time

The overall mean Global Cognitive Reaction Time scores in the FGP condition (M=-0.059, SD=0.49) and UGP condition (M=0.061, SD=0.49) did not significantly differ, as indicated by

Condition being non-significant. Time was found to be a significant predictor of Global Cognitive Reaction Time, F(7, 80) = 2.349, p = 0.031. However, Bonferroni corrected post hoc tests revealed no significant differences between assessments. Neither Condition or Regulator Type were found to be significant predictors of Global Cognitive Reaction Time, indicating that similar global reaction times were produced between conditions and glucoregulatory groups.

5.3.3.5.3 Global Cognitive Performance (Accuracy and Reaction Time)

The overall mean Global Cognitive Performance scores in the FGP condition (M=0.017, SD=0.49) and UGP condition (M=-0.33, SD=0.49) did not significantly differ, as indicated by Condition being non-significant. Global Cognitive Performance was not predicted by either Time or the Condition by Time interaction, indicating that the participants did not perform significantly differently between or throughout the FGP and UGP conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups did not display significantly different levels of Global Cognitive Performance.

5.3.3.6 Cognitive Task Performance Comparisons

5.3.3.6.1 CRT and Merged tasks (CRT aspect within Merged task)

5.3.3.6.1.1 CRT vs. Merged Percent Correct

As expected, Task was found to be a significant predictor of Percent Correct; F(1, 600) = 111.011, p < 0.001, with participants achieving a significantly higher percent of correct responses for the CRT task (M=98.18, SD=1.29) compared to Merged CRT performance (M=95.67, SD=1.85). No significant interaction between Task and any other predictor was found, indicating that this performance difference was consistent across different levels of Condition, Time and Regulator Type.

5.3.3.6.1.2 CRT vs. Merged Reaction Time

Reaction Time significantly differed between tasks as indicated by Task being a significant predictor; F(1, 1071) = 230.334, p < 0.001, with participants reacting significantly quicker during the CRT task (M=332.32, SD=10.62) compared to Merged CRT performance (M=358.97, SD=11.04). Interestingly, the two-way interaction between Condition and Task was also significant; F(1, 673) = 23.378, p < 0.001. As shown in Figure 5.9, post hoc tests also revealed an effect of Task where participants were significantly slower during Merged CRT performance compared to the CRT task within both conditions. Post hoc tests also revealed that Merged CRT performance was significantly faster during the UGP condition compared to the FGP condition, whilst no significant differences between conditions were found for the CRT task (see Figure 5.9).

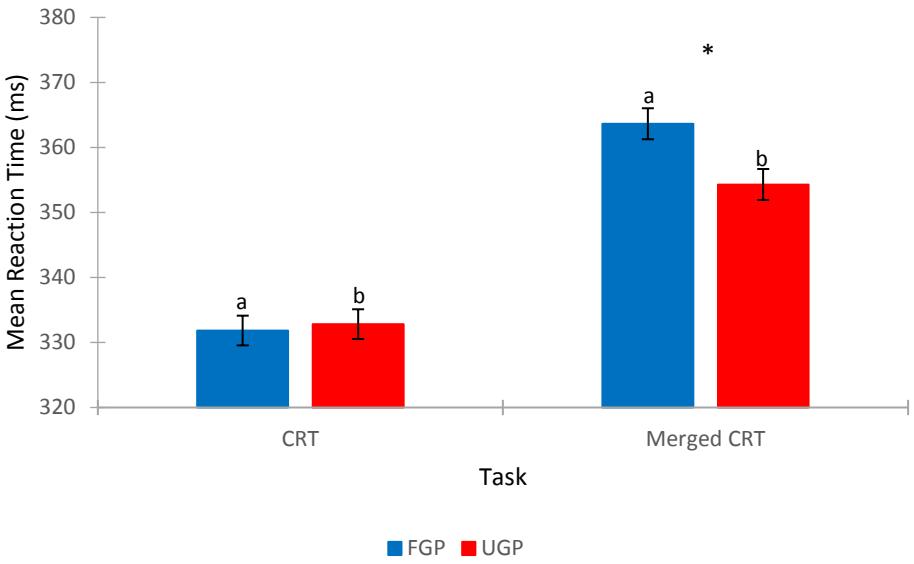


Figure 5.9: Reaction Time condition means for CRT and Merged cognitive tasks

Values represent estimated marginal means for CRT and Merged cognitive tasks within each condition. An asterisk (*) indicates a significant difference between conditions for a cognitive task. Condition means that share the same letter are significantly different from one another within condition. Error bars represent standard error of the mean. The figure indicates that reaction times were significantly quicker for the CRT task compared to Merged CRT performance within both conditions, whilst participants were significantly quicker during Merged CRT performance in the UGP condition compared to the FGP condition. No significant differences between conditions were found for the CRT task.

5.3.3.6.2 RVIP and Merged tasks (RVIP aspect within Merged task)

5.3.3.6.2.1 RVIP vs. Merged Percent Correct

Task was a significant predictor of Percent Correct; $F(1, 839) = 115.502$, $p < 0.001$, with participants achieving a higher percent of correct responses on the RVIP task (M=85.80, SD=7.49) compared to Merged RVIP performance (M=78.76, SD=8.03). The three-way interaction between Condition, Task and Regulator Type was significant for Percent Correct; $F(1, 640) = 6.901$, $p = 0.009$. Post hoc tests revealed that the good glucose regulators performed significantly better than poor glucose regulators on Merged RVIP performance within the FGP condition, suggesting that the good glucoregulatory group benefited more from the LGI meals under an increased cognitive load (see Figure 5.10). No significant differences were seen between glucoregulatory groups for the RVIP task within either condition. Neither glucoregulatory group performed significantly differently on either task between conditions.

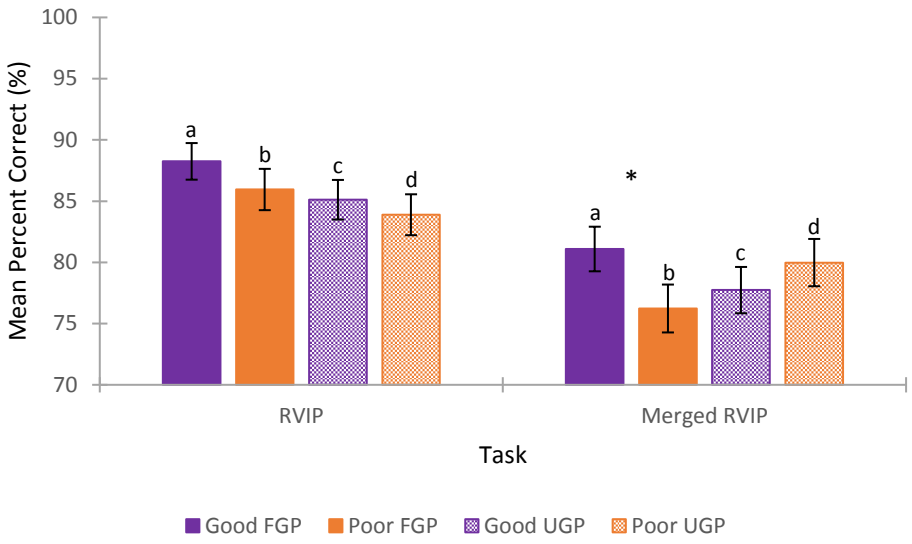


Figure 5.10: Percent Correct glucoregulatory group means for RVIP and Merged tasks within each condition

Values represent estimated marginal means for good and poor regulators within each condition (above = FGP, below = UGP). There were 20 good and 20 poor glucose regulators. An asterisk (*) indicates a significant difference between glucoregulatory groups on a cognitive task within the FGP condition only. Glucoregulatory group means that share the same letter are significantly different from one another within condition. Error bars represent standard error of the mean. The figure indicates that good glucose regulators were significantly more accurate during Merged RVIP performance compared to the poor glucose regulators within the FGP condition. No significant differences between glucoregulatory groups were found for the RVIP task in either condition. Both glucoregulatory groups were significantly less accurate during Merged RVIP performance compared to the RVIP task within both conditions.

5.3.3.6.2.2 RVIP vs. Merged Reaction Time

As expected, Task was found to be a significant predictor of Reaction Time; $F(1, 1009) = 21.12$, $p < 0.001$, with participants reacting significantly quicker on the RVIP task (M=419.90, SD=13.95) compared to Merged RVIP performance (M=436.46, SD=17.25). The two-way interaction between Task and Regulator Type was also significant for Reaction Time; $F(1, 542) = 44.47$, $p < 0.001$. Post hoc tests revealed that good glucose regulators were significantly quicker during the RVIP task compared to Merged RVIP performance but this was not seen for the poor glucose regulators (see Figure 5.11). Post hoc tests also revealed that poor glucose regulators were significantly quicker during Merged RVIP performance compared to good glucose regulators.

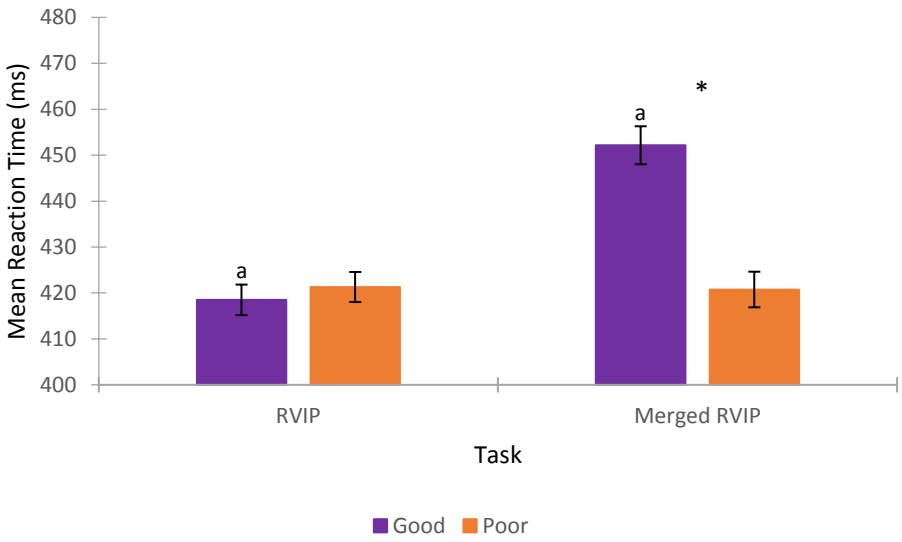


Figure 5.11: Reaction Time glucoregulatory group means for RVIP and Merged tasks

Values represent estimated marginal means for good and poor regulators within each cognitive task. There were 20 good and 20 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a cognitive task. Task means that share the same letter are significantly different from one another for good glucose regulators only. Error bars represent standard error of the mean. The figure indicates that poor glucose regulators were significantly quicker during Merged RVIP performance compared to good glucose regulators. Good glucose regulators were significantly faster on the RVIP task compared to Merged RVIP performance. Poor glucose regulators did not produce significantly different reaction times between cognitive tasks.

5.3.4 Subjective Mood

5.3.4.1 Alertness

The overall mean Subjective Alertness levels in the FGP condition (M=59.37, SD=8.65) and UGP condition (M=63.06, SD=8.65) did not significantly differ, as indicated by Condition being non-significant. Time was a significant predictor of Subjective Alertness, F(4, 80) = 2.818, p = 0.03. However, post hoc tests revealed no significant differences in subjective alertness between any assessment time points. The Condition by Time interaction did not significantly predict Subjective Alertness, indicating similar alertness levels throughout both conditions. No significant differences were found between glucoregulatory groups as indicated by Regulator Type being non-significant.

5.3.4.2 Anxiety

The overall mean Subjective Anxiety levels in the FGP condition (M=33.75, SD=9.52) and UGP condition (M=37.99, SD=9.52) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Subjective Anxiety, indicating that the participants did not report significantly different

anxiety levels between and within the FGP and UGP conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar anxiety levels throughout testing.

5.3.4.3 Contentment

The overall mean Subjective Contentment levels in the FGP condition (M=71.29, SD=12.35) and UGP condition (M=71.74, SD=12.28) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Subjective Contentment, indicating that the participants did not report significantly different contentment levels between and within the FGP and UGP conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar contentment levels throughout testing.

5.3.5 Subjective Hunger, Fullness and Sleepiness (HFS)

5.3.5.1 Hunger

The overall mean Subjective Hunger levels in the FGP condition (M=36.92, SD=15.81) and UGP condition (M=37.47, SD=15.81) did not significantly differ, as indicated by Condition being non-significant. Interestingly, overall Subjective Hunger did not significantly differ throughout the day as indicated by Time being non-significant. However, the interaction between Time and Regulator Type was significant for Subjective Hunger, $F(4, 80) = 3.21$, $p = 0.017$. Post hoc tests revealed that poor glucose regulators were significantly hungrier 95 minutes after lunch (14:00) compared to 15 minutes post lunch (12:15), which suggests poor glucose regulators found the lunch meals to be less satiating across the mid-afternoon (see Figure 5.12). Good and poor glucose regulators did not report significantly different levels of subjective hunger at any assessment time point.

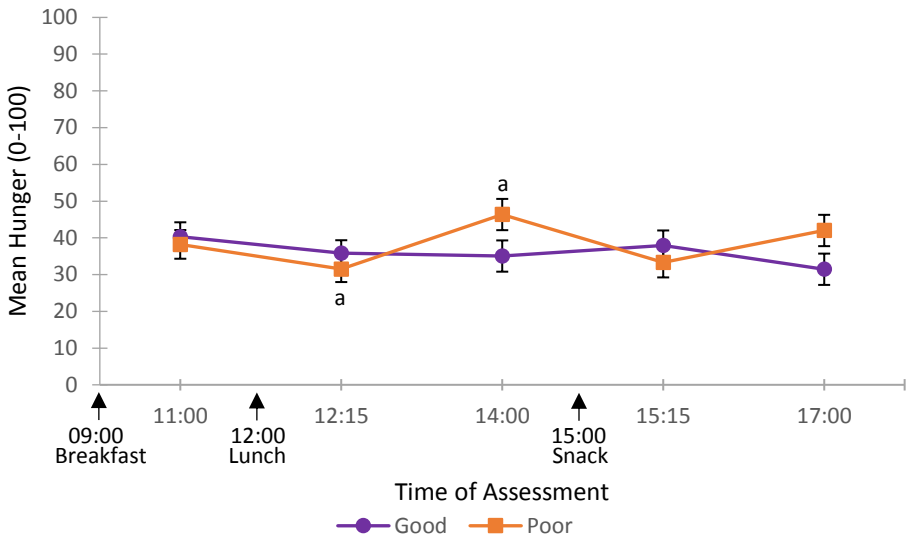


Figure 5.12: Mean Subjective Hunger for glucose regulators at each testing session

Values represent estimated marginal means for good (above) and poor glucose regulators (below) at each testing session across conditions. There were 12 good and 12 poor glucose regulators. Test sessions that share the same letter are significantly different from one another for the poor glucoregulatory group. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure shows that poor glucose regulators were significantly hungrier in the mid-afternoon compared to 15 minutes after lunch consumption. Good glucose regulators did not significantly differ between sessions.

5.3.5.2 Fullness

The overall mean Subjective Fullness levels in the FGP condition (M=55.25, SD=14.02) and UGP condition (M=52.18, SD=14.02) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Subjective Fullness, indicating that the participants felt similar levels of Subjective Fullness between and within the FGP and UGP conditions across the day. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar Subjective Fullness throughout testing.

5.3.5.3 Sleepiness

The overall mean Subjective Sleepiness levels in the FGP condition (M=49.53, SD=11.54) and UGP condition (M=46.98, SD=11.54) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Subjective Sleepiness, indicating that the participants felt similar levels of Subjective Sleepiness between and within the FGP and UGP conditions across the day. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar Subjective Sleepiness throughout testing.

5.3.6 Summary of findings

The collective findings from the analysis of glycaemic, cognitive performance and subjective mood measures can be found in Table 5.2.

Table 5.2: Summary of glycaemic, cognitive performance and subjective mood findings.

Measure	Benefits for FGP	Benefits for UGP	Benefits for good glucose regulation	Benefits for poor glucose regulation
Glycaemic Response	A lower glycaemic response after every meal.	None observed.	A lower glycaemic response average overall.	None observed.
Cognitive Performance	None observed.	Good regulators produced quicker LM reaction times 30 minutes post lunch. Faster overall Merged CRT reaction times.	Higher Merged accuracy average in FGP only. Higher Merged RVIP accuracy average in FGP only.	Faster overall Merged RVIP reaction times.
Subjective Mood	None observed.	None observed.	None observed.	None observed.
Subjective ratings of Hunger, Fullness and Sleepiness	None observed.	None observed.	None observed.	None observed.

5.4 Discussion

The primary aim of this study was to investigate whether there were any significant differences in cognitive performance between a favourable glucose profile (FGP) and an unfavourable glucose profile (UGP) in a healthy sample. The second aim was to investigate whether glucose tolerance status within a healthy sample predicted cognitive performance. This study also aimed to investigate whether any significant differences in subjective mood ratings were found between the FGP and UGP conditions in a healthy sample. The final aim was to investigate whether glucose tolerance status within a healthy sample predicted subjective mood ratings.

5.4.1 The Glycaemic Response

It was important to measure the glycaemic response and examine whether the two meal profiles (FGP vs. UGP) produced measurably different glycaemic responses in the study population. This allows any significant differences in cognitive performance and subjective mood measures to be examined and interpreted with the added context of the glycaemic response data. To summarise, the glycaemic response profiles were as expected with a significantly higher glycaemic response occurring after each meal in the UGP condition. The comparison between glucoregulatory groups indicated that the poor glucose regulators produced a significantly higher glycaemic response average than the good glucose regulators, although no significant differences at a specific assessment time were observed. Interestingly, there appears to be two key differences in the glycaemic response between the present sample and the one examined in Study 1. Firstly, the overall glycaemic response throughout both conditions is noticeably lower, with the present sample's range being between 3.25- and 6mmol/L, whereas the range in Study 1 was between 4.4- and 7.5mmol/L. Secondly, the glycaemic response seen in the FGP condition appears to be consistently more stable following each meal. The more stable glycaemic response in the FGP condition observed in the present study resulted in more significant differences between conditions following the snack compared to Study 1. Taken together, these findings reflect better glucose tolerance, which is typical in younger adults compared to the elderly (Basu et al., 2003; DeFronzo, 1981; Shimokata, 1991). The larger age range used in Study 1 (18-65 years) compared to Study 2 (18-25 years) included older participants, some of whom likely have poorer glucose tolerance within the NGT range. The glycaemic response differences across studies 1 and 2 provide further evidence that even within the NGT range, researchers may want to consider differences between good and poor glucose regulators (Lamport et al., 2009). The observation that poor glucose regulators within the sample showed higher mean glucose concentrations may be explained by slightly (but not significantly) higher glycaemic responses occurring following one or more of the meals. It is plausible that the poor glucose regulators may have slightly reduced insulin sensitivity and secretion compared to the good glucose regulators, although this reduction may not be enough to statistically impact the glycaemic response after each meal. It should be noted that whilst this explanation is logically sound it cannot be confirmed as insulin was not measured in the present study. Finally, the glycaemic response shown following the breakfast meals are remarkably similar between conditions in Study 1,

whereas the glycaemic response following the UGP breakfast was significantly higher compared to the FGP breakfast in the present study. It should be noted that whilst this could be due to a younger sample with comparably better glucose tolerance being examined in the present study than in Study 1, it is also plausible that the lack of a standardised test meal in Study 1 could provide a potential explanation for the differences in glycaemic response following breakfast between Study 1 and the present study.

5.4.2 Cognitive Performance

The initial analysis of the cognitive data returned limited significant differences between conditions, with the majority of findings being significant differences between assessment time points for the reaction time aspect of the cognitive tasks. Typically, these findings indicated that the slowest reaction times were recorded during the post lunch assessments and before snack consumption, which is indicative of a post-lunch dip (Monk, 2005). However, it should be noted that no concurrent significant differences in accuracy were observed between assessments for any of the cognitive tasks. This suggests that participants are taking longer to respond during the afternoon sessions in order to maintain accuracy across the day. Interestingly, reaction times tended to become quicker across the morning sessions before lunch for the CRT and RVIP tasks, indicating improvements in psychomotor function and attention across the morning. This can be compared to Micha et al. (2011) who found improved performance on measures of attention across the morning after HGI meal consumption. However, the difference between these findings is that the improvements in attention in the present study appear to be due to general meal consumption, with neither LGI or HGI meals being significantly more beneficial than the other. One potential reason for this difference could be the matching of energy and macronutrients between meals. For example, Micha et al. (2011) failed to match energy and macronutrient contents between conditions, whilst energy and macronutrients were fully matched between test meals in the present study. These methodological differences may have contributed to the mixed results between the previous research and the current study here. It has been previously indicated that fat and protein share a negative relationship with postprandial glucose rise (Jenkins et al., 1981). Animal models have supported this by indicating that insulin responses to glucose in rats were augmented 3-fold and 1.5-fold by the addition of whey protein or oleic acid respectively (Gunnarsson et al, 2006). Thus, it is plausible that Micha et al. (2011) identified the HGI condition as more beneficial to cognitive performance due to confounding effects of macronutrient mismatching between conditions. It is therefore logical that the current study avoided these confounding effects by accurately matching the energy and macronutrient content between test meals, which could explain why neither condition appears more beneficial to attention than the other.

The reaction time component of the LM task appeared to get progressively quicker throughout the day, suggesting the presence of practise effects. This may also reflect differences in task difficulty between the LM task and the other three cognitive tasks. For example, the CRT task presents a simple choice of whether a target appears on the left or right of the screen, whereas the LM task involves the remembrance and recognition of several letters as well as

the order in which they were presented before a choice is made. If participants found the LM task more difficult to comprehend and perform then the progressively quicker reaction times throughout the day may reflect an increasing understanding of the task brought about by repeated exposure to the task (Bell et al., 2018). It was also found that good glucose regulators produced significantly slower reaction times thirty minutes after lunch consumption during the FGP condition compared with the UGP condition, whilst poor glucose regulators did not significantly differ between conditions at any assessment. This finding may reflect a combination between GI differences in the lunch meals and the underlying effects of glucose tolerance. For example, a higher GI meal typically produces a higher glycaemic response and a faster supply of glucose into the bloodstream (Jenkins et al., 1981), which is observed here (see Section 5.3.2). Better glucose tolerance is associated with a faster glucose uptake rate of cellular tissue and more efficient transport of glucose across the BBB (see Chapter 2). Thus, it is plausible that the good glucose regulators produce a quicker mean reaction time after the HGI lunch, in comparison to the LGI lunch, due to a quicker availability of glucose from the HGI lunch and a more efficient transport of that glucose into the brain. Thus, the poor glucose regulators may not be displaying significant differences between conditions due to a less efficient utilization of glucose, brought about by comparatively poorer glucose tolerance. In line with this theory, a general pattern emerged whereby quicker LM reaction times were observed throughout the majority of the UGP condition for good glucose regulators, whilst the reverse pattern was evident for the poor glucoregulatory group. This further suggests that the combination of a faster glucose availability from the HGI meals and the presence of comparatively better glucose tolerance benefited cognitive performance. This pattern also suggests that poor glucose regulators stand to gain more cognitive benefits from the consumption of LGI meals. This finding supports the proposal that glycaemic interventions are most beneficial to cognitive performance in those with poorer glucose tolerance (Lamport et al., 2009).

The impact of task difficulty was further considered through the statistical comparison of both the CRT and RVIP tasks with their respective aspects of the Merged task. The results indicated that participants were significantly slower during Merged CRT performance compared with the CRT task, but accuracy did not significantly differ. This suggests that participants took longer to process the information presented in the Merged task due to increased task difficulty when compared to the CRT task. The comparison between the RVIP and Merged tasks indicated that both glucoregulatory groups were significantly more accurate on the RVIP task compared to Merged RVIP performance. This further suggests that increasing task difficulty through the introduction of another performance component (i.e. additionally identifying target location) along with identifying numerical target strings is adversely affecting performance. Interestingly, good glucose regulators were significantly more accurate than poor glucose regulators for Merged RVIP performance in the FGP condition, whilst no differences were observed during the UGP condition. This was also the case during initial analysis of general Merged task performance, before task comparisons were carried out. These findings support previous research that identifies complex task performance as particularly sensitive to the facilitative effects of glucose (Benton et al., 1994; Foster et al., 1998; Reay, Kennedy, Scholey, 2006; Riby et al., 2006; Sunram-Lea et al., 2002; Parker & Benton, 1995). Furthermore, this finding can be compared to Cooper et al. (2015) who found that participants performed better

on more complex levels of a stroop test following LGI meal consumption. However, the fact that only good glucose regulators in the current sample appear to benefit from the FGP meals, whilst the poor glucose regulators do not, further suggests that glucose tolerance has a moderating effect on the relationship between glycaemic response and complex task performance here. In other words, even though a LGI meal provides a steadier supply of glucose into the bloodstream (Jenkins et al., 1981), glucose tolerance affects how efficient that glucose is subsequently utilised. As aforementioned, a higher insulin resistance has been associated with a less efficient transport of glucose across the BBB (see Chapter 2). Previous research has indicated an increase in glucose uptake by the brain during a complex visual-spatial motor task in humans (Haier et al., 1992), whilst rat studies have observed decreases in neural interstitial glucose levels proportional to the difficulty of a maze task (McNay et al., 2000). Any dysfunctions to the transport of glucose across the BBB may result in a less efficient supply of glucose to the brain during cognitive tasks, which have been shown to deplete neural glucose levels proportional to their complexity. Thus, good glucose regulators may be performing significantly better than poor glucose regulators due to a combination of a steadier supply of glucose from the FGP meals and a more efficient transport of this glucose across the BBB into the brain during the more complex Merged task. Although, the comparison between RVIP and Merged tasks also revealed that good glucose regulators reacted significantly slower overall than poor glucose regulators during Merged RVIP performance. Therefore, it is also plausible that an accuracy-reaction time trade-off is occurring for the good glucose regulators where they may be taking longer than poor glucose regulators to give a response in order to improve accuracy during Merged RVIP performance (Bogacz et al., 2010; Fitts, 1966; Reed, 1973; Wickelgren, 1977; Wood & Jennings, 1976).

To summarise, analysis of the cognitive data returned limited significant differences in cognitive performance between conditions and glucoregulatory groups. In line with previous research, general improvements in measures of attention across the morning were observed (Micha et al., 2011). It has been suggested that the energy and macronutrient matching between test meals in the present study may explain why general meal consumption, rather than a particular condition, is beneficial for subsequent cognitive performance. It is likely that the overall good glucose tolerance status of the sample means that underlying mechanisms associating glucose tolerance and cognitive performance have not been largely compromised (see Chapter 2, Section 2.4). Good functionality of these mechanisms means that subsequent cognitive impairments are unlikely in both glucoregulatory groups examined. Although, the data does provide some evidence that even minimal differences in mechanisms such as glucose transport efficiency across the BBB may affect cognitive performance under increased cognitive load such as the Merged and LM tasks here. Poorer performance is likely due to the increased uptake of neural glucose during more complex cognitive tasks (Hair et al., 1992; McNay et al., 2000) and an inability to replenish these decrements efficiently in the presence of comparatively poorer glucose tolerance. It has also been suggested that better accuracy during Merged RVIP performance may be due to good glucose regulators committing an accuracy-reaction time trade-off, where they sacrifice reaction time to improve accuracy (Bogacz et al., 2010; Fitts, 1966; Reed, 1973; Wickelgren, 1977; Wood & Jennings, 1976). However, it should be noted that no qualitative measurement of task strategy was taken so this theory, whilst plausible, cannot be confirmed by the current data. By extending the

previous single meal testing paradigm to a three meal investigation, this study has highlighted that the previously reported improvements in cognitive domains such as attention across the morning, following breakfast consumption, do not always continue into the afternoon. Indeed, the current findings suggest that cognitive performance worsens post lunch, indicative of a post-lunch dip (Monk, 2005), before gradually improving during the late afternoon. The exception to this was the measure of executive function (LM task), which showed gradual improvements in reaction time throughout the test day. This is likely due to practise effects as participants gained greater proficiency in a more complex cognitive task, requiring less time to maintain accuracy with each assessment.

5.4.3 Subjective Mood

There were no significant differences between conditions or glucoregulatory groups for alertness, anxiety or contentment. This suggests that an acute manipulation of the glycaemic response via dietary GI/GL manipulation does not have a significant impact on subjective mood in an ostensibly healthy sample. This finding is consistent with previous research which has also reported no mood differences between LGI and HGI meal conditions in acute settings (Cooper et al., 2015; Micha et al., 2010). This comparison is interesting as methodological discrepancies exist between the studies, whilst the findings appear consistent. For example, Micha et al. (2010) did not match test meals for energy and macronutrients whereas the current study did. Given that energy and macronutrient content was matched between test meals in the present study, as well as the cross-over research design, the differences in glycaemic response between conditions can be more readily attributed to GI variation (Jenkins et al., 1981). With larger glycaemic responses occurring in the UGP condition, it can be reasonably inferred that larger insulin responses would follow (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997). Previous research has indicated that acutely elevated insulin levels are associated with the increased production of the neurotransmitter norepinephrine (Trulson & Himmel, 1985), which has been shown to increase arousal, alertness and vigilance whilst also increasing restlessness and anxiety (Aston-Jones, 1981; Oken, Salinksy & Elsas, 2006; Southwick et al., 1999; Wise & Stein, 1969). However, the good glucose tolerance status of the sample suggests they have good insulin sensitivity, which would result in naturally lower levels of insulin needing to be secreted as well as faster insulin absorption by cellular tissue (James et al., 1988; Kahn & Pessin, 2002; Watson et al., 2004; Wang et al., 2014). Therefore, it is likely that insulin levels are not elevated in amplitude or duration enough to produce significant mood differences between conditions even with significantly higher glycaemic and insulin responses occurring throughout the UGP condition.

The lack of significant differences between good and poor glucose regulators could also be explained through the overall good glucose tolerance status of the sample. Poorer glucose tolerance has been associated with increased neuroinflammation (see Chapter 2), which can have deleterious effects on neuronal structure and function such as dopaminergic neurons (Benatti et al., 2016; De Felice & Ferreira, 2014; Le Moal & Simon, 1991). There is evidence that a reduction of dopamine in mood mediating areas of the brain such as the amygdala is associated with depression (D'Haenen & Bossuyt, 1994; Ebert et al., 1996; Klimeck et al., 2002;

Shah et al., 1997). Given the overall good glucose tolerance status of the sample, the increased negative affect and depressive symptoms associated with chronic neuroinflammation in those with poorer glucose tolerance (see Chapter 2) are less likely to be present due to minimal, if any, neuroinflammation. Taken together, these findings suggest that an acute manipulation of the glycaemic response may not have a significant impact on subjective mood in an ostensibly healthy sample with a good glucose tolerance status. This finding is of interest as it indicates that the previously reported lack of mood differences between two single meal conditions, based upon the GI concept, is still present when investigated across three consecutive meals in a healthy sample. Given that divergent glycaemic profiles did occur between conditions, this provides evidence for a potential moderating effect of glucose tolerance in the relationship between glycaemic response and subjective mood.

5.4.4 Subjective ratings of Hunger, Fullness and Sleepiness

Results from measurements of subjective hunger, fullness and sleepiness returned minimal significant findings. In fact, the only significant finding from this section of analysis was that poor glucose regulators were significantly hungrier 95 minutes after lunch compared to only 15 minutes after lunch, whilst good glucose regulators did not significantly differ throughout the day. The increased hunger levels seen 95 minutes after lunch for the poor glucose regulators is likely attributed to increasing levels of the ghrelin (hunger stimulating hormone) being released by the ghrelinergic cells in response to stomach emptying (Dickson et al., 2011; Inui et al., 2004; Meier & Gressner, 2004; Sakata & Sakai, 2010; Schwartz et al., 2000). If ghrelin concentrations are affecting subjective hunger, then differences in gut motor activity between glucoregulatory groups may explain why only the poor glucose regulators significantly vary throughout the day. Previous research has indicated that the results of an OGTT in healthy subjects can vary with the phase of normal upper gut motor activity occurring at the point of glucose ingestion (Thompson et al., 1982). The authors suggest that this difference is the result of different rates of delivery of the glucose solution to the absorptive surface of the small intestine. In context of the present study, poor glucose regulators may have a higher upper gut motor activity than the good glucose regulators post lunch, which could result in a quicker emptying of the stomach. In turn, a quicker emptying of the stomach could lead to a faster increase in ghrelin production, which may present itself as the increased hunger reported by the poor glucose regulators 95 minutes post lunch. Conversely, a slower gut motor activity in the good glucoregulatory group, and a subsequently slower stomach emptying rate, could result in slower ghrelin production, which would explain why good glucose regulators report similar subjective hunger levels throughout the day.

The lack of significant findings between conditions for hunger, fullness and sleepiness could be considered surprising due to the test meals not being matched in weight. Indeed, the FGP meal weights were 465g, 440g and 440g for the breakfast, lunch and snack respectively. Whereas, the UGP meal weights were 291g, 534g, and 183g respectively. The clear differences in weight suggest that the test meals may stretch the stomach to different degrees upon consumption, with heavier and larger meals stretching it more. An increased stretching of the stomach following a meal is associated with reduced ghrelin production, reduced stimulation

of the hypothalamic cells and subsequent reductions in hunger (Inui et al., 2004; Meier & Gressner, 2004; Sakata & Sakai, 2010). Considering no significant differences occur even in light of the weight differences between meals, it is plausible that insulin sensitivity and secretion may be affecting subjective hunger and fullness ratings. For example, a slower rate of insulin-mediated glucose transport into cellular tissue could lead to increased hunger, which has been associated with a hyperglycaemic state (Srinivasan & Ramarao, 2007; Triplitt, 2012; Nakamura, 1962). Given that the present sample were all of good glucose tolerance and young age, it is plausible that their good insulin sensitivity leads to fast insulin-mediated glucose transport into cellular tissue within both conditions. If this were the case, then any polyphagia that is associated with hyperglycaemia may not occur due to the hyperglycaemic state being too short in length for a significant impact on subjective hunger to take place in either condition (Srinivasan & Ramarao, 2007; Triplitt, 2012; Nakamura, 1962).

Interestingly, it has also been shown that acute increases in circulating insulin levels are associated with reduced ghrelin levels (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014). As aforementioned (see Section 5.4.1), subtle differences in insulin sensitivity and secretion between glucoregulatory groups is likely but these differences may not be great enough to significantly impact circulating ghrelin levels in either group. If this were the case, then it may explain the lack of significant differences in both hunger and fullness between glucoregulatory groups. Furthermore, elevated ghrelin levels have been associated with lower sleepiness ratings and shorter sleep duration (Taheri et al., 2004). This finding also suggests that subtle differences in insulin sensitivity and secretion within the NGT sample may not significantly impact ghrelin concentrations. Taken together, the findings indicate that those with lower insulin sensitivity and secretion, characteristic of poorer glucose tolerance (e.g. T2DM), may display higher levels of polyphagia due to slower glucose uptake rates and ghrelin dysregulation. Given that the present sample all had NGT, it is likely that only subtle differences in insulin sensitivity and secretion exist between participants, which may not alter glucose uptake rates or ghrelin regulation enough to significantly impact the self-report measures.

5.5 Conclusions

In conclusion, this study demonstrates that minimal differences in cognitive performance and mood were observed between glycaemic conditions in a healthy young sample across three consecutive meals. However, the meal profiles did produce significantly different glycaemic responses throughout the day. Specifically, the FGP condition was associated with a lower and more consistent glycaemic response compared to the UGP condition. This finding is in line with the concept of GI, as the HGI meals produced higher glycaemic responses as would be expected (Jenkins et al., 1981). There were limited differences in cognition between glucoregulatory groups, which is likely due to the overall good glucose tolerance status of the sample. Although, there was some evidence that LGI meals may be more beneficial to cognitive performance during more complex tasks, in the presence of better glucose tolerance. It is suggested that whilst participants may vary in glucose tolerance measures such as insulin sensitivity and secretion within the NGT range, these differences are likely to be

subtle in nature. It is likely that underlying mechanisms which associate glucose tolerance and cognition have suffered little to no injury in presence of good glucose tolerance (see Chapter 2, Section 2.4). Thus, it is proposed that the minimal significant differences between conditions and glucoregulatory groups is due to the efficient operation of the underlying mechanisms associating glucose tolerance and cognition, even in the presence of divergent glycaemic responses. Taken together, the results suggest that glucose tolerance may have a moderating role in the relationship between glycaemic response and cognition. The next question of interest is whether a poorer glucose tolerance sample (e.g. T2DM) will display cognitive and mood impairments in either glycaemic condition. This may be more likely to occur as poorer glucose tolerance is associated with increasing cognitive impairment and negative affect via the dysfunction of multiple underlying mechanisms (see Chapter 2).

Chapter 6

Utilising GI: An Investigation of Glycaemia and Cognition in Type 2 Diabetes (Study 3)

6.1 Introduction

The previous study in this thesis reported minimal significant differences in cognitive performance between glycaemic conditions in an ostensibly healthy sample (see Chapter 5). It has been suggested that the mechanisms associating glucose tolerance and cognitive performance may not be compromised in healthy individuals, which could explain the mixed findings in such samples even when glycaemic response profiles significantly differ between conditions (see Chapter 2, Section 2.4). Furthermore, previous research has suggested that those with poorer glucose tolerance may be more likely to display significant cognitive differences between glycaemic conditions (Lamport et al., 2009). Interestingly, the current literature utilising GI/GL to investigate the relationship between the acute glycaemic response and cognitive performance in T2DM is scarce. In fact, the review in this thesis (see Chapter 2) only identified two studies which had used a T2DM sample in acute settings (i.e. Lamport et al., 2013; Papanikolaou et al., 2006). Lamport et al. (2013) reported that the T2DM group displayed significantly poorer verbal memory than a NGT group following a meal omission condition. Whereas, Papanikolaou et al. (2006) found that those with T2DM had significantly better verbal memory and working memory during the LGI condition compared to the HGI condition. Taken together, these findings support the proposal that those with poorer glucose tolerance may benefit more from glycaemic interventions compared to healthy individuals (Lamport et al., 2009). There is a current lack of research that has investigated the relationship between glycaemic response and cognitive performance across multiple meals in a T2DM sample (see Chapter 2). Given that humans consume multiple meals throughout the day, the relationship between glycaemic response and cognition in those with poorer glucose tolerance (e.g. T2DM) across the day warrants investigation. Thus, a primary aim of the present study was to investigate whether any significant differences in cognitive performance are observed between two multi-meal glycaemic conditions in those with T2DM. The use of a glucose composite score is also used here in order to split comparatively good and poor glucose regulators within the T2DM sample, as the severity of T2DM can greatly vary on an individual basis (WHO, 2016). This allows an investigation of whether T2DM severity is associated with cognitive performance within the context of the multi-meal testing paradigm.

Similar to cognitive performance research, there are many cross-sectional and longitudinal studies that examine the relationship between glucose tolerance and subjective mood outcomes in T2DM samples, but few acute investigations (see Chapter 2, Section 2.3). The use of longitudinal or cross-sectional research designs means that variations in glucose tolerance have generally been tracked through the measurement of HbA1c levels over time (higher level = poorer glucose tolerance). The findings from these studies were largely consistent with higher HbA1c levels being associated with more negative outcomes such as anxiety, depression and distress (see Chapter 2, Section 2.3). However, the relationship between the acute glycaemic response and mood outcomes in T2DM appears to be more limited. Indeed,

the review in this thesis only identified three studies with this methodology (i.e. Greenwood et al., 2003; Pais et al., 2007; Sommerfield et al., 2004). The findings from these studies indicated that acute periods of hyperglycaemia are associated with increased rates of negative mood outcomes such as anger and anxiety (Pais et al., 2007; Sommerfield et al., 2004), although no significant mood differences were observed between a meal consumption and omission condition (Greenwood et al., 2003). However, it should be noted that none of the three acute studies implemented the concept of GI/GL to investigate the glycaemic response and mood outcomes. Instead, two studies used glycaemic clamp techniques to maintain euglycaemia and hyperglycaemia conditions (Pais et al., 2007; Sommerfield et al., 2004) whilst the other provided participants with a meal or placebo but did not monitor the glycaemic response (Greenwood et al., 2003). Thus, the relationship between glycaemic response and mood in T2DM remains largely unexplored in acute settings and warrants further investigation. Therefore, this study aimed to investigate whether any significant mood differences were observed between two multi-meal glycaemic conditions (FGP vs. UGP) in those with T2DM. As shown in the previous study (Chapter 5), minimal mood differences occurred between conditions and glucoregulatory groups in a healthy sample, which is likely due to the uncompromised mechanisms associating glucose tolerance and subjective mood (see Chapter 2). However, a T2DM sample will have a characteristically greater glycaemic variability (ADA, 2016) which has been previously associated with increased negative affect (Penckofer et al., 2012) along with increased injury to underlying mechanisms that associate glucose tolerance and mood (see Chapter 2). These previous findings along with the proposal that those with poorer glucose tolerance may benefit more from glycaemic interventions than healthy individuals (Lamport et al., 2009) suggest that the current T2DM sample are more likely to show sensitivity to the meal manipulations for subjective mood outcomes than healthy samples (see Chapters 4 & 5). Finally, good and poor glucose regulators were also compared on mood outcomes to investigate whether glucose tolerance status within the T2DM range predicted subjective mood.

To summarise, the previous study in this thesis (Chapter 5) investigated the relationship between glycaemic response, cognitive performance and mood in healthy individuals. The findings indicated minimal significant differences in cognitive performance and mood between glycaemic conditions even in the presence of divergent glycaemic response profiles. It is suggested that this is due to the efficient operating of the underlying mechanisms that associate glucose tolerance and cognition (see Chapter 2, Section 2.4). Therefore, the present study aimed to investigate the relationship between glycaemic response, cognitive performance and mood in a T2DM sample. Given that T2DM has been associated with the dysfunction of multiple underlying mechanisms such as glucose transport across the BBB (see Chapter 2, Section 2.4), it is plausible that this population are more likely to show cognitive differences between the two glycaemic conditions. Finally, the same glucose composite score method used in previous studies within this thesis was also employed to compare good and poor glucose regulators within the T2DM sample. Typically, HbA1c levels are repeatedly measured in longitudinal research involving T2DM samples in order to investigate changes in HbA1c and any associated cognitive outcomes. However, in an acute setting the use of a glucose composite score has been proposed to have more ecological validity than the use of a single glycaemic parameter (e.g. fasting glucose levels) as it encompasses the whole glycaemic

response rather than a single section (Lamport et al., 2009). The aims of the present study are described below.

6.1.1 Summary of aims

Aim 1: To investigate whether there are any significant differences in cognitive performance between a favourable glucose profile (FGP) and an unfavourable glucose profile (UGP) within a T2DM population.

Aim 2: To investigate whether glucose tolerance status within a T2DM population predicts cognitive performance.

Aim 3: To investigate whether any significant differences in subjective mood ratings are found between the FGP and UGP within a T2DM population.

Aim 4: To investigate whether glucose tolerance status within a T2DM population predicts subjective mood ratings.

6.1.2 Study Hypotheses

Hypothesis 1: The FGP condition will be associated with improved cognitive performance across the day compared to the UGP condition.

Hypothesis 2: Good glucose regulators will display significantly better cognitive performance across the day compared to poor glucose regulators within the sample.

Hypothesis 3: The FGP condition will be associated with improved subjective mood ratings across the day compared to the UGP condition.

Hypothesis 4: Good glucose regulators will display significantly better subjective mood across the day compared to poor glucose regulators within the sample.

6.2 Method

6.2.1 Power analysis

A prior power analysis was conducted using Gpower 3.1 to determine the sample size required. Assuming an effect size of d = 0.44 (Papanikolaou et al., 2006) with a statistical power of 0.8 (which was a conservative selection based upon a hard to reach clinical sample) and an alpha level of 0.05, 42 participants were deemed sufficient to detect cognitive performance differences between conditions in a T2DM sample. Ultimately, it was not feasible to recruit the 42 participants required based upon the power calculation due to a difficult to reach clinical sample, although, 25 participants did take part. However, it is likely that the effect size reported by Papanikolaou et al. (2006) is an underestimate of the effect size that can be expected in the present study, which involved a higher number of testing points. In

other words, increasing the number of testing points increases the power of the present study. Therefore, it was deemed that the 25 participants recruited would be sufficient to detect differences between glycaemic conditions in the present T2DM sample.

6.2.2 Recruitment

Opportunistic sampling involved sending emails to relevant group mailing lists, the handing out of fliers and posters, as well as posting on the University of Reading campus and Reading town notice boards. All participants were recruited from the local population within the county of Berkshire.

6.2.3 Participants

Twenty-five adults with a self-reported medical diagnosis of T2DM were recruited through local advertisement at the University of Reading and surrounding areas. These were 17 males and 8 females, with a mean age of 56.9 years (SD = 7.8) (see section 6.3.1 for full details). Inclusion criteria were aged between 40–70 years old. All participants were self-reported non-smokers, with no relevant food intolerances or allergies.

Exclusion criteria were a medical diagnosis of any form of cancer, or any condition that could affect glucose metabolism apart from diabetes (e.g. anaemia and pregnancy). Prescribed anti-depressants was also an exclusion criterion as these drugs have the potential to impact glucose metabolism (Deuschle, 2013; Himmerich, Minkwitz & Kirkby, 2015) and cognitive function (Hindmarch, Kimber & Cockle, 2000; Knegtering, Eijck & Huijsman, 1994). Finally, any participant that was a self-reported professional athlete was excluded, as these individuals could have improved glucose uptake due to skeletal muscular adaptations such as increased GLUT4 glucose transporter expression (Goodyear & Kahn, 1998). All exclusion criteria were checked through self-reporting on the health & lifestyle medical questionnaire, which participants completed and returned by email before a screening session could be arranged (Appendix H).

6.2.4 Study design

The present study followed a counterbalanced, randomised, crossover design using the same two experimental conditions implemented in Studies 1 and 2. The order in which a participant took part in the experimental conditions was obtained by entering participant numbers into an online researcher randomiser (Urbaniak & Plous, 2013). To allow for potential dropouts, twice the number of required participants was entered into the randomiser prior to the study. This resulted in twelve of the sample beginning with the FGP condition, whilst the other thirteen took part in the UGP condition initially. For the order of participation see Appendix E. The two conditions were (i) Favourable Glycaemic Profile (FGP) - a LGI diet consisting of a breakfast, lunch and afternoon snack and (ii) Unfavourable Glycaemic Profile (UGP) - a HGI diet consisting

of a breakfast, lunch and afternoon snack. The independent variables were Condition, Time and Regulator Type, whilst the dependent variables were Cognitive Performance, Glycaemic Response, Subjective Mood, Hunger, Fullness and Sleepiness ratings.

6.2.5 Nutritional manipulations

The conditions were identical to those described in Chapter 4, Section 4.5.2. All macronutrient compositions along with GI/GL values can be found in sections 4.2.5.1 and 4.2.5.2.

6.2.6 Outcome variables

6.1.6.1 Glycaemic Response

Interstitial glucose was measured with the exact same procedure and frequency as Study 2 (see Chapter 5, section 5.2.6.1).

6.1.6.2 Cognitive Performance

The procedure and frequency of cognitive performance assessment was identical to that of Study 2 (see Chapter 5, section 5.2.6.2).

6.1.6.3 Self-report measures (questionnaires)

The procedure and frequency of subjective mood and hunger, fullness and sleepiness evaluations were identical to that of Study 2 (see Chapter 5, section 5.2.6.3).

6.2.7 Procedure

6.1.7.1 Screening

The screening procedure was identical to Study 2 (see Chapter 5, section 6.2.7.1). Once screening was completed, the test days were arranged, the first of which was required to be a least one week following the screening session.

6.1.7.2 Testing

6..7.2.1 Prior dietary instructions

Prior dietary instructions were identical to those of Study 2 (see Chapter 5, Section 5.2.7.2.1).

6..7.2.2 Day sessions

Testing day procedure was identical to that implemented throughout Study 2 (see Chapter 5, section 5.2.7.2.2).

6.2.8 Payment

The participant payment amounts were standardised in line with the Hugh Sinclair Unit of Human Nutrition guidelines. All participants received £50 per visit, giving a total of £100 paid to each. This amount was considered sufficient to financially remunerate participants for their time and any incurred travel expenses.

6.2.9 Ethical approval

This study received a favourable opinion for conduct from the School of Psychology Research Ethics Committee (SREC), the University of Reading Ethics Committee (UREC) and the East of Scotland Research Ethics Service (EoSRES). Evidence of ethical approval can be found in Appendix N. The clinical trials ID number for this study is NCT03360604.

6.2.10 Statistical analysis

Statistical analysis and reporting were identical to that implemented throughout Study 2 (see Chapter 5, section 5.2.10).

6.3 Results

6.3.1 Participant characteristics

The characteristics of seventeen male and eight female participants are shown in Table 6.1. All participants were within the 40-70 years age range.

Table 6.1: Participant Characteristics (n = 25, 17 male, 8 female)

Variable	M	SD	Min	Max
Height (cm)	173.9	9.7	158	192
Weight (kg)	92.9	19.9	57.9	137.2
BMI (kg/m^2)	30.6	5.3	19.6	42
Age (years)	56.9	7.8	40	70
Fasting Glucose (mmol/L)	8.44	2.65	4.4	18.2

Note. Fasting Glucose value was obtained at screening.

*M = Mean, SD = Standard Deviation, Min = Minimum value, Max = Maximum value.

6.3.2 Glycaemic Response

An initial t test indicated that baseline glucose values did not significantly differ between the FGP condition (M=8.49, SD=2.54) and UGP condition (M=8.38, SD=2.84) at the start of the day; t(24) = 0.477, p = 0.638. Condition approached significance for Glycaemic Response; F(1, 64) = 2.978, p = 0.089, but post hoc tests revealed no significant differences between conditions. The interaction between Condition and Time was significant for Glycaemic Response; F(21, 380) = 4.416, p < 0.001. Bonferroni corrected post hoc tests revealed that the glycaemic response was significantly higher in the UGP condition at 210, 225, 240, 270, 300, 420, 450 and 480 minutes (all: p<0.05), indicating that longer periods of hyperglycaemia were experienced after the consumption of HGI lunch and snack compared with the LGI lunch and snack (see Figure 6.1).

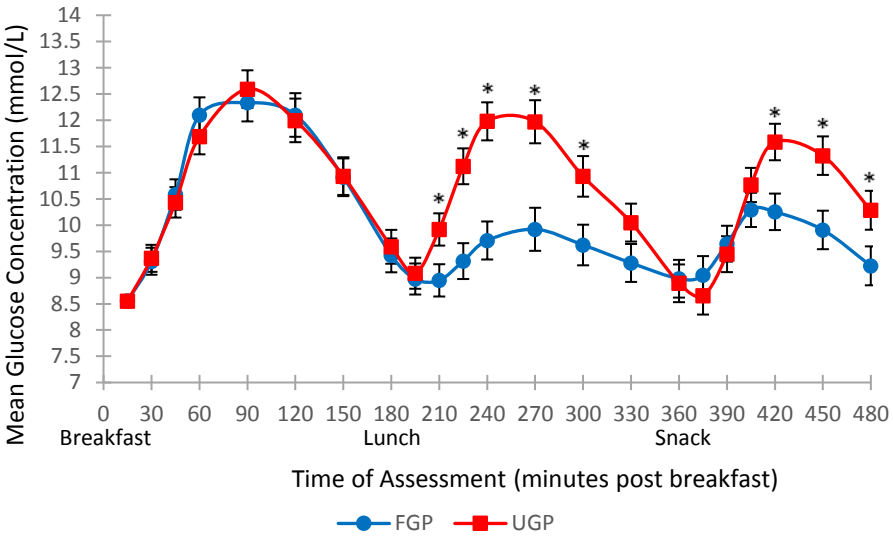


Figure 6.1: The interaction between Condition and Time for Glycaemic Response

Data points represent estimated marginal means across all participants at each time point. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Error bars represent standard error of the mean. Baseline glucose (0 minutes) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure indicates that a significantly higher glycaemic response was produced in the UGP condition following both the lunch and snack compared to the FGP condition.

In regards to glucose tolerance status, poor glucose regulators produced a significantly higher glycaemic response average (M=9.44mmol/L, SD=1.24) compared to good glucose regulators (M=11mmol/L, SD=1.19), indicated by Regulator Type being a significant predictor, F(1, 74) = 19.711 p < 0.001. The two-way interaction between Time and Regulator Type was also significant for Glycaemic Response; F(21, 380) = 3.09, p < 0.001. Bonferroni corrected post hoc tests revealed that the glycaemic response was significantly higher for poor glucose regulators than good glucose regulators at 90, 120, 150, 180, 195, 210, 225, 240, 270, 300, 330, 360, 375, 390, 405, 420, 450 and 480 minutes (all: p<0.05), indicating longer periods of hyperglycaemia that is characteristic of poorer glucose tolerance (see Figure 6.2).

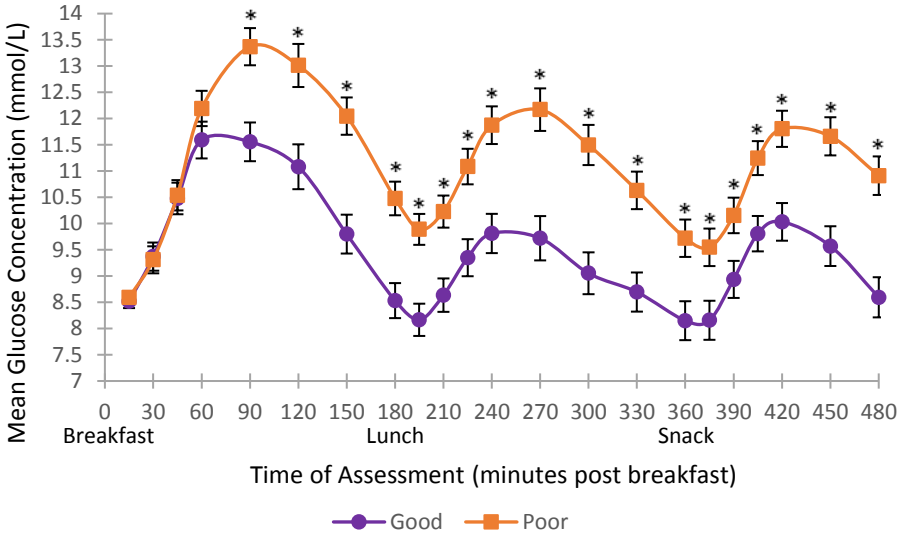


Figure 6.2: The interaction between Time and Regulator Type for Glycaemic Response

Data points represent estimated marginal means across glucoregulatory groups at each time point. An asterisk (*) indicates a significant difference between glucoregulatory groups at an assessment time point. Error bars represent standard error of the mean. Baseline glucose (0 minutes) was included as a covariate in the linear mixed model, hence its exclusion from the figure. The figure shows that poor glucose regulators produced a significantly higher glycaemic response than good glucose regulators after lunch and snack consumption.

6.3.3 Cognitive Performance

6.3.3.1 Choice Reaction Time Task

For raw RT data, there were an initial 26,583 trials with an associated correct response. A total of 16 trials had a RT of under 100ms and were removed. A further 309 trials were identified as potential outliers due to having a Z score of 3.29 or higher and were removed before calculating session means. Histograms demonstrating this outlier process step by step can be found in Appendix O. This task did not contain a false positives element, so all resulting session means were included in Accuracy and RT analyses.

6.3.3.1.1 CRT Accuracy

Neither Time or Regulator Type were found to significantly predict Accuracy on the CRT task, indicating that the performance of participants did not significantly differ between sessions or glucoregulatory groups. The overall mean CRT Accuracy scores in the FGP condition (M=59.07, SD=0.76) and UGP condition (M=59.04, SD=0.76) did not significantly differ, as indicated by Condition being non-significant. However, the two-way interaction between Condition and Time approached significance; $F(7, 50) = 2.06$, $p = 0.065$. Post hoc tests indicated that participants were significantly more accurate at 12:30 in the UGP condition compared with the

FGP condition (see Figure 6.3). No significant differences between sessions were found within either condition.

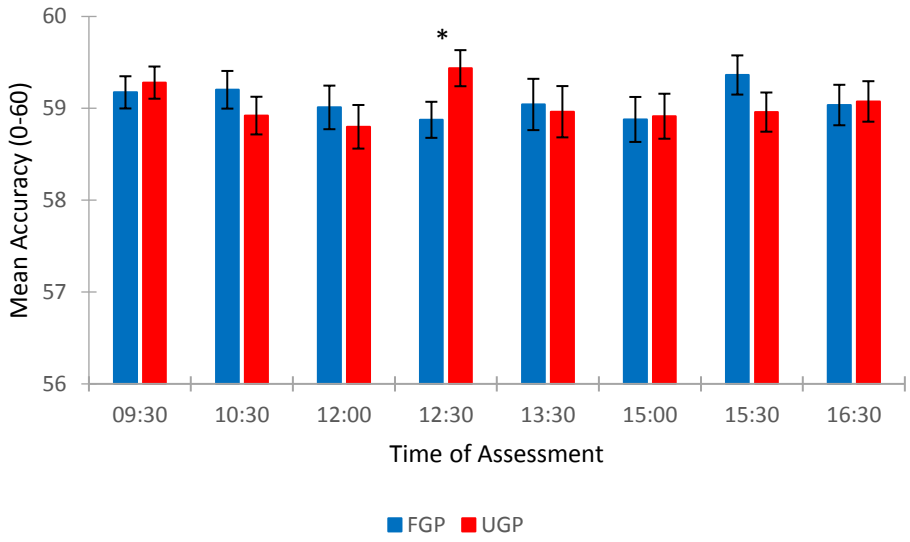


Figure 6.3: The interaction between Condition and Time for CRT task Accuracy

Values represent estimated marginal means across all participants at each time point. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Error bars represent standard error of the mean. The figure shows that participants were significant more accurate on the CRT task during the UGP condition 30 minutes post lunch compared to the FGP condition.

6.3.3.1.2 CRT Reaction Time

The overall mean CRT task Reaction Times in the FGP condition (M=364.61, SD=26.25) and UGP condition (M=368.97, SD=26.27) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting CRT Reaction Time, $F(7, 50) = 1.982$, $p = 0.076$. However, Bonferroni correct post hoc tests revealed no significant differences between any assessment time points. The Condition by Time interaction did not significantly predict CRT Reaction Time indicating that the participants performed similarly between and within the FGP and UGP conditions. Regulator Type was found to be a significant predictor of CRT Reaction Time; $F(1, 55) = 5.81$, $p = 0.019$, with good glucose regulators (M=377.57, SD=29.74) being significantly slower than poor glucose regulators (M=356, SD=28.37).

6.3.3.2 Rapid Visual Information Processing Task

For raw RT data, there were an initial 8,257 trials with an associated correct response. A total of 3 trials had a RT of under 100ms and were removed. A further 115 trials were identified as potential outliers due to having a Z score of 3.29 or higher. However, these 115 trials were not deemed to be sufficient enough outliers after consultation with histograms and consideration

of the task's short timeframe (100-800ms), and thus were not removed from subsequent analysis. Histograms demonstrating this outlier process step by step can be found in Appendix O. No session means for any participant had an associated false positives rate over the 33% cut off, so the removal of session means was not required before analyses.

6.3.3.2.1 RVIP Accuracy

The overall mean RVIP Accuracy scores in the FGP condition (M=18.39, SD=1.07) and UGP condition (M=18.40, SD=1.07) did not significantly differ, as indicated by Condition being non-significant. However, the interaction between Condition and Time was significant for RVIP task Accuracy; F(7, 50) = 2.356, p = 0.037. Post hoc tests revealed that participants were significantly less accurate immediately before lunch at 12:00 in the UGP condition compared with the FGP condition (see Figure 6.4). No significant differences between assessments were found within either condition. Furthermore, no significant differences were seen between glucoregulatory groups as indicated by Regulator Type being non-significant.

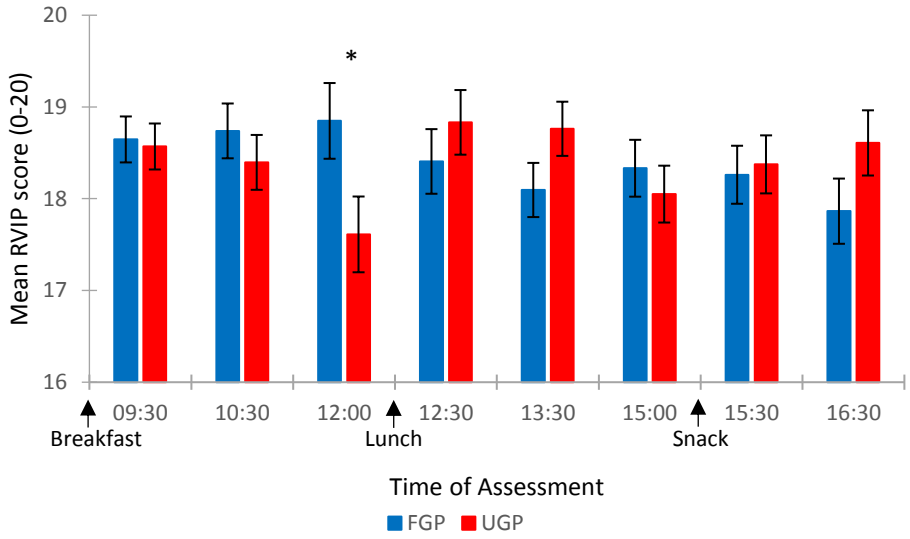


Figure 6.4: The interaction between Condition and Time for RVIP task Accuracy

Values represent estimated marginal means across all participants at each time point. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Error bars represent standard error of the mean. The figure indicates that participants were significantly more accurate on the RVIP task immediately before lunch in the FGP condition compared to the UGP condition.

6.3.3.2.2 RVIP Reaction Time

The overall mean RVIP task Reaction Times in the FGP condition (M=403.45, SD=26.69) and UGP condition (M=405.54, SD=26.71) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting RVIP Reaction Time, F(7, 50)

= 1.89, p = 0.091. However, Bonferroni correct post hoc tests revealed no significant differences between any assessment time points. The Condition by Time interaction did not significantly predict RVIP Reaction Time indicating that the participants performed similarly between and within the FGP and UGP conditions. No significant differences were seen between glucoregulatory groups, as indicated by Regulator Type being non-significant.

6.3.3.3 Merged Task

For raw RT data, there were an initial 5,995 trials with an associated correct response. No trials had a RT of under 100ms, nor did any trial have a Z score of 3.29 or higher. A histogram demonstrating this can be found in Appendix O. No session means for any participant had an associated false positives rate over the 33% cut off, so the removal of session means was not required before analyses.

6.3.3.3.1 Merged Accuracy

The overall mean Merged task Accuracy scores in the FGP condition (M=13.55, SD=2.94) and UGP condition (M=13.35, SD=2.94) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Accuracy on the Merged task, indicating that the performance of participants did not significantly differ between or within conditions. Regulator Type approached significance for predicting Merged task Accuracy; F(1, 54) = 2.945, p = 0.092, but post hoc tests revealed no significant differences between glucoregulatory groups.

6.3.3.3.2 Merged Reaction Time

The overall mean Merged task Reaction Times in the FGP condition (M=570.36, SD=40.54) and UGP condition (M=569.76, SD=38.04) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting Merged task Reaction Time, F(7, 42) = 2.058, p = 0.07. However, Bonferroni corrected post hoc tests revealed no significant differences between assessments across conditions. The three-way interaction between Condition, Time and Regulator Type was found to significantly predict Merged task Reaction Time, F(7, 42) = 3.583, p = 0.004. However, Bonferroni corrected post hoc tests revealed no significant differences between sessions for either glucoregulatory group within either condition. Post hoc tests also indicated that no significant differences were found between glucoregulatory groups at any assessment point within either condition. Furthermore, Merged task Reaction Time did not significantly differ between conditions at any assessment time point for either glucoregulatory group.

6.3.3.4 Letter Memory Task

For raw RT data, there were an initial 5,078 trials with an associated correct response. No trials had a RT of under 100ms. A total of 35 trials were identified as potential outliers due to having a Z score of 3.29 or higher and were removed before calculating session means. Histograms demonstrating this outlier process step by step can be found in Appendix O. This task did not contain a false positives element, so all resulting session means were included in Accuracy and RT analyses.

6.3.3.4.1 LM Accuracy

The overall mean LM task Accuracy scores in the FGP condition (M=10.73, SD=2.43) and UGP condition (M=10.56, SD=2.41) did not significantly differ, as indicated by Condition being non-significant. LM task Accuracy significantly varied throughout the test day as indicated by Time being a significant predictor, $F(7, 50) = 4.75$, $p < 0.001$. As shown in Figure 6.5, participants were significantly less accurate in the morning at 09:30 than in the afternoon at 12:30, 13:30, 15:00 and 16:30. Given that performance steadily rises from the morning sessions into the afternoon test sessions, this may reflect practise effects from repeated exposure to the cognitive task.

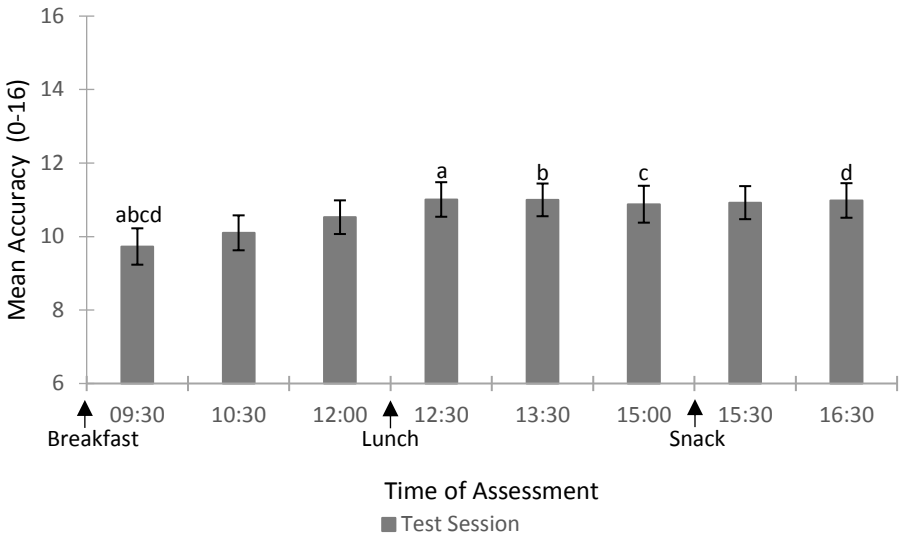


Figure 6.5: Mean LM task Accuracy for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. The figure shows that participants were significantly less accurate in the earliest morning session compared to three sessions post lunch, and one session post snack consumption (16:30). Performance generally increases into the afternoon sessions, suggesting potential practise effects.

The three-way interaction between Condition, Time and Regulator Type was also found to significantly predict LM task Accuracy, F(7, 50) = 3.107, p = 0.008. As shown in Figure 6.6, post hoc tests revealed that poor glucose regulators produced significantly fewer correct responses at 09:30 than at 12:30 and 16:30 in the FGP condition. The accuracy of poor glucose regulators in the FGP condition gradually increased across the morning but dropped during the mid-afternoon. Accuracy began to increase again after snack consumption at 15:00, suggesting that meal consumption improved performance. Post hoc tests also revealed that good glucose regulators produced significantly more correct responses at 16:30 than at 10:30 in the UGP condition (see Figure 6.6). Furthermore, neither glucoregulatory group significantly differed between conditions at any assessment time point. However, there appears to be an interesting general pattern that for the good glucose regulators improved accuracy is seen in the FGP condition, whilst the reverse pattern is seen for poor glucose regulators. For example, 13:30 shows this pattern, suggesting that the FGP and UGP lunch had differential effects on performance dependent upon glucoregulatory group (see Figure 6.6).

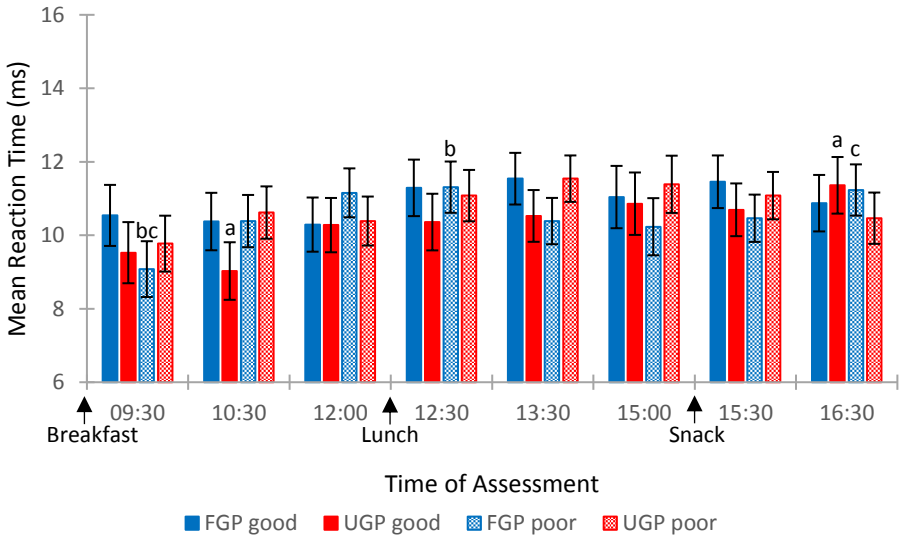


Figure 6.6: LM task Accuracy glucoregulatory group means within each condition

Values represent estimated marginal means for good and poor regulators at assessment time points within each condition (above = good regulators, below = poor regulators). There were 12 good and 13 poor glucose regulators. Test sessions sharing the letter are significantly different from each other within condition for a glucoregulatory group. Error bars represent standard error of the mean. The figure shows that both glucoregulatory groups produced significantly more correct responses on the LM task during the afternoon compared to the morning sessions. There is also a general pattern where the performance of poor glucose regulators appears to benefit more from the UGP condition, whilst good glucose regulators benefit more from the FGP condition.

6.3.3.4.2 LM Reaction Time

Neither Regulator Type or Time were found to significantly predict LM task Reaction Time, indicating that glucoregulatory groups reacted at similar speeds between and within conditions. The overall mean LM task Reaction Times in the FGP condition (M=2950.17,

SD=464.11) and UGP condition (M=2959.44, SD=464.54) did not significantly differ, as indicated by Condition being non-significant. However, the three-way interaction between Condition, Time and Regulator Type was significant for LM task Reaction Time; F(7, 50) = 2.349, p = 0.037. Post hoc tests revealed that poor glucose regulators were significantly faster in the FGP condition at 12:30 compared with the UGP condition, suggesting the FGP and UGP lunch meals had differential effects on performance for the poor glucoregulatory group (see Figure 6.7). Post hoc tests also revealed that poor glucose regulators were significantly faster at 12:30 than at 10:30 in the FGP condition but were significantly slower at 12:30 than at 16:30 in the UGP condition (see Figure 6.7). There appears to be a general pattern in the data where good glucose regulators are slower to respond in the FGP condition compared to the UGP condition, whilst the reverse is seen for poor glucose regulators. An example of this can be seen at the 12:30 assessment (see Figure 6.7).

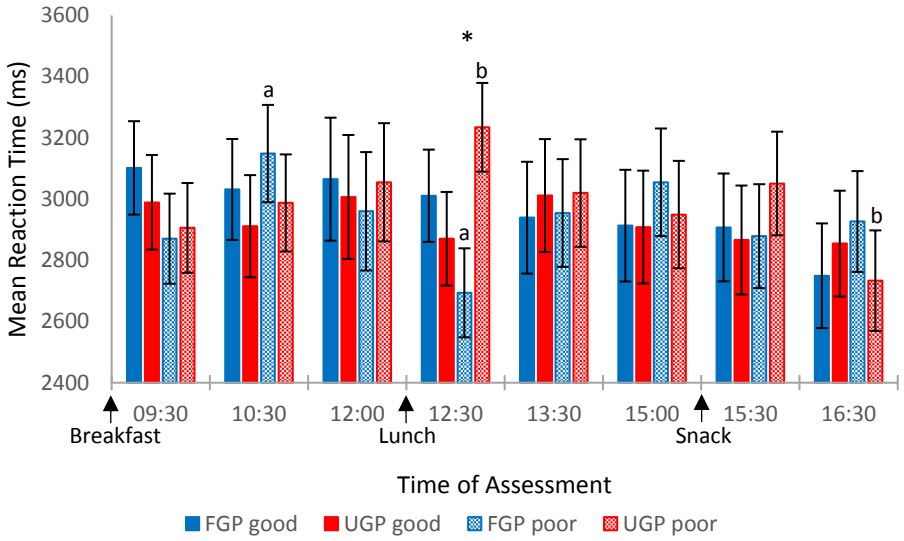


Figure 6.7: LM task Reaction Time condition means within glucoregulatory groups

Values represent estimated marginal means for good and poor regulators at assessment time points within each condition (above = good regulators, below = poor regulators). There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between conditions at an assessment time point within the poor glucoregulatory group only. Test sessions sharing the same letter are significantly different from each other for a glucoregulatory group within condition. Error bars represent standard error of the mean. The figure shows that good glucose regulators reacted at similar speeds throughout the day in both conditions, whilst poor glucose regulators significantly differed between the morning and afternoon in both conditions. Poor glucose regulators were significantly slower to react 30 minutes after lunch in the UGP condition compared to the FGP condition, suggesting the meals had differential effects on performance. A general pattern where good glucose regulators react slower in the FGP condition compared to the UGP condition, and the reverse pattern for the poor glucose regulators, can also be seen.

6.3.3.5 Global Cognition

6.3.3.5.1 Global Cognitive Accuracy

The overall mean Global Cognitive Accuracy scores in the FGP condition (M=0.052, SD=0.41) and UGP condition (M=0.009, SD=0.41) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting Global Cognitive Accuracy, $F(7, 50) = 2.111$, $p = 0.059$. However, Bonferroni corrected post hoc tests revealed no significant differences between assessments across conditions. The two-way interaction between Condition and Time and was found to significantly predict Global Cognitive Accuracy, $F(7, 50) = 2.771$, $p = 0.016$. Post hoc tests revealed that participants were significantly more accurate 30 minutes after compared to immediately before lunch in the UGP condition, whereas no significant differences between assessments were observed for the FGP condition. Global Accuracy greatly declines following the UGP breakfast, which suggests this meal is impairing performance across the morning. Interestingly, Global Accuracy increases in the mid-morning following FGP breakfast consumption and slowly declines into the afternoon, which suggests the FGP breakfast was beneficial at sustaining performance for a longer time frame compared to the UGP breakfast. Global Accuracy significantly increases following UGP lunch consumption, whilst a small decrease is seen after the FGP lunch. Finally, performance appears to increase in both conditions immediately after snack consumption but Global Accuracy decreases in the final test session during the FGP condition whilst increasing in the UGP condition, suggesting a delayed beneficial effect of the UGP snack to performance (see Figure 6.8).

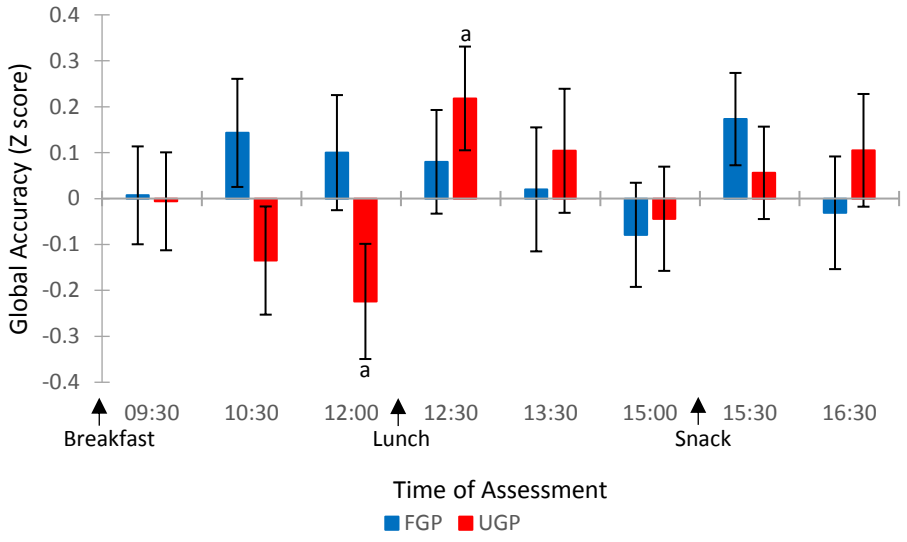


Figure 6.8: The interaction between Condition and Time for Global Cognitive Accuracy

Values represent estimated marginal means across all participants at each time point. Test sessions sharing the same letter are significantly different from one another within the UGP condition only. Error bars represent standard error of the mean. The figure shows that performance greatly decreased across the morning following the UGP breakfast, while being improved and relatively sustained across the morning in the FGP condition. The UGP lunch appears to benefit Global Accuracy, whereas performance appears unaffected by FGP lunch consumption. Performance improves following snack consumption in both conditions, although this effect seems to be more delayed in the UGP condition.

6.3.3.5.2 Global Cognitive Reaction Time

For clarity, a positive Z score represents a faster reaction time whilst a negative Z score represents a slower reaction time in this section of analysis. The overall mean Global Cognitive Reaction Time scores in the FGP condition (M=0.031, SD=0.54) and UGP condition (M=-0.037, SD=0.54) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting Global Cognitive Reaction Time, $F(7, 50) = 1.867$, $p = 0.095$. However, Bonferroni corrected post hoc tests revealed no significant differences between assessments across conditions. The two-way interaction between Condition and Time also approached significance for Global Cognitive Reaction Time; $F(7, 50) = 2.108$, $p = 0.06$. Post hoc tests revealed that participants were significantly slower immediately before lunch at 12:00 than in the afternoon at 16:30 in the UGP condition, whereas no significant differences were observed throughout the FGP condition. (see Figure 6.9). Interestingly, reaction times become slower across the morning in both conditions, although the greater decline is seen following the UGP breakfast. Reaction times become quicker following both lunch meals, but only the FGP lunch is followed by reaction times above the sample average, indicating better performance in this condition during the mid-afternoon. Although participants are performing below the sample average for the majority of the afternoon in the UGP condition, there are gradual improvements following UGP lunch consumption, which continue after snack consumption, suggesting the presence of a beneficial second meal cognitive effect from the UGP lunch (see Figure 6.9). Reaction times improve following snack consumption in both conditions, indicating snack consumption was beneficial for performance (see Figure 6.9).

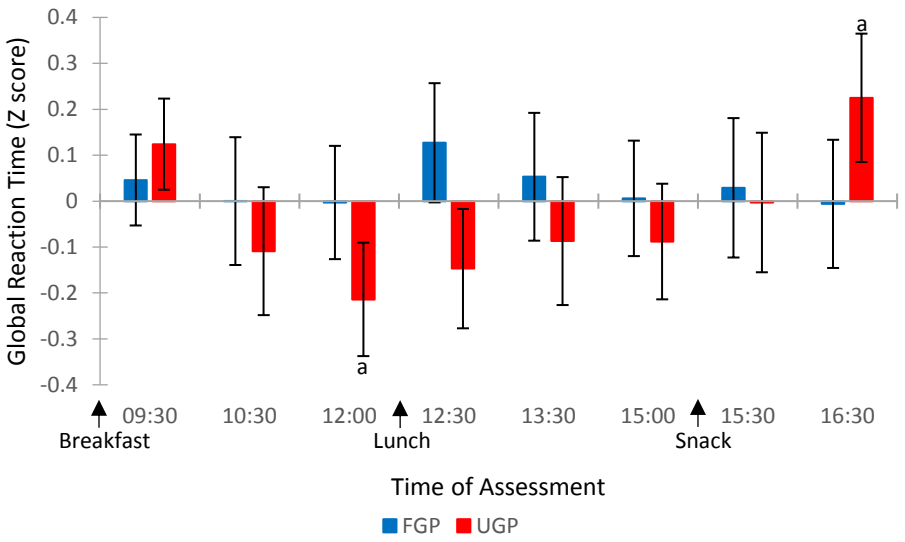


Figure 6.9: The interaction between Condition and Time for Global Cognitive Reaction Time

Values represent estimated marginal means across all participants at each time point. Test sessions sharing the same letter are significantly different from one another within the UGP condition only. Error bars represent standard error of the mean. The figure shows that a greater decline in reaction time occurred in the UGP condition following breakfast consumption. Reaction times improved post lunch in both conditions but were only above the sample average in the FGP condition. Both snacks were followed by faster reaction times. General improvements are observed after the UGP lunch, which continue after snack consumption, suggesting the presence of a second meal cognitive effect.

6.3.3.5.3 Global Cognitive Performance (Accuracy and Reaction Time)

The overall mean Global Cognitive Performance scores in the FGP condition (M=0.063, SD=0.41) and UGP condition (M=-0.029, SD=0.41) did not significantly differ, as indicated by Condition being non-significant. Time approached significance for predicting Global Cognitive Performance, $F(7, 50) = 2.099$, $p = 0.061$. However, Bonferroni corrected post hoc tests revealed no significant differences between assessments across conditions. The two-way interaction between Condition and Time and was found to significantly predict Global Cognitive Performance, $F(7, 50) = 2.5$, $p = 0.028$. Post hoc tests revealed participants performed significantly better immediately before lunch at 12:00 in the FGP condition compared to the UGP condition (see Figure 6.10). Post hoc tests also revealed that participants performed significantly better at 16:30 compared to immediately before lunch at 12:00 in the UGP condition (see Figure 6.10). Interestingly, Global Cognitive Performance appears to greatly decline following the UGP breakfast whilst being relatively sustained across the morning after the FGP breakfast, which suggests the meals have differential effects on performance. Both lunch meals are followed by an initial increase and then a gradual decline in performance across the mid-afternoon assessments. Finally, snack consumption in both conditions is followed by improved performance, although the largest improvement in the UGP condition is seen at 16:30, indicating a delayed beneficial effect of the UGP snack to Global Cognitive Performance (see Figure 6.10).

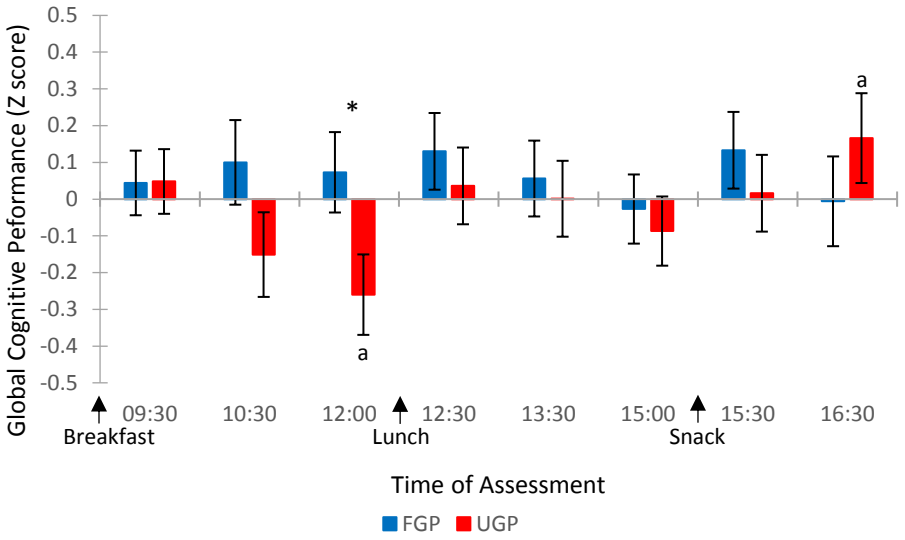


Figure 6.10: The interaction between Condition and Time for Global Cognitive Performance

Values represent estimated marginal means across all participants at each time point. An asterisk (*) indicates a significant difference between conditions at an assessment time point. Test sessions sharing the same letter are significantly different from one another within the UGP condition. No significant differences between sessions within the FGP condition were found. Error bars represent standard error of the mean. The figure shows that performance greatly declines after UGP breakfast consumption but is sustained across the morning following the FGP breakfast. Both lunch meals are followed by an initial improvement in performance before a gradual decline across the mid-afternoon assessments. Both snacks are followed by improved performance but the figure suggests a delayed beneficial effect of the HGI snack is present.

6.3.3.6 Cognitive Task Performance Comparisons

6.3.3.6.1 CRT and Merged tasks (CRT aspect within Merged task)

6.3.3.6.1.1 CRT vs. Merged Percent Correct

As expected, Task was found to be a significant predictor of Percent Correct; $F(1, 434) = 46.825$, $p < 0.001$, with participants achieving a significantly higher percent of correct responses for the CRT task (M=96.01, SD=1.05) compared to Merged CRT performance (M=92.89, SD=2.32), indicating a performance drop between tasks. Post hoc tests following the Condition by Task interaction approaching significance; $F(1, 321) = 2.731$, $p = 0.099$, indicated no significant differences between conditions occurred for either task. However, there is a noticeable pattern where performance was remarkably similar between conditions on the CRT task, whilst better performance was observed during the UGP condition for Merged CRT performance (see Figure 6.11). This suggests the higher cognitive load of the Merged task may make it more sensitive to glycaemic interventions. Post hoc tests also indicated an effect of Task where participants achieved a higher percentage of correct responses on the CRT task, compared to Merged CRT performance, in both conditions (see Figure 6.11).

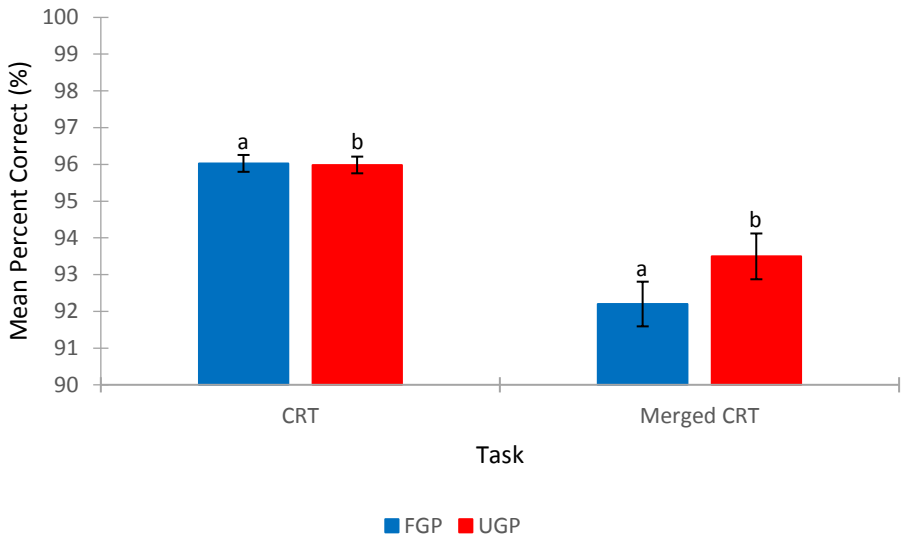


Figure 6.11: Percent Correct condition means for CRT and Merged cognitive tasks

Values represent estimated marginal means for CRT and Merged cognitive tasks within each condition. An asterisk (*) indicates a significant difference between cognitive tasks within a condition. No significant differences between conditions for either task was found. Error bars represent standard error of the mean. The figure indicates that better performance was observed for the CRT task across both conditions. It also appears that better Merged CRT performance was seen in the UGP condition compared to the FGP condition.

The two-way interaction between Task and Regulator Type was significant in predicting Percent Correct; $F(1, 330) = 11.22$, $p = 0.001$. Post hoc tests revealed an effect of Task with good and poor glucose regulators being significantly more accurate on the CRT task compared to Merged CRT performance (see Figure 6.12). Post hoc tests also revealed that good glucose regulators were significantly more accurate than poor glucose regulators during Merged CRT performance, suggesting that the compromise of underlying mechanisms that associate glucose tolerance and cognitive performance become more apparent under an increased cognitive load (see Figure 6.12).

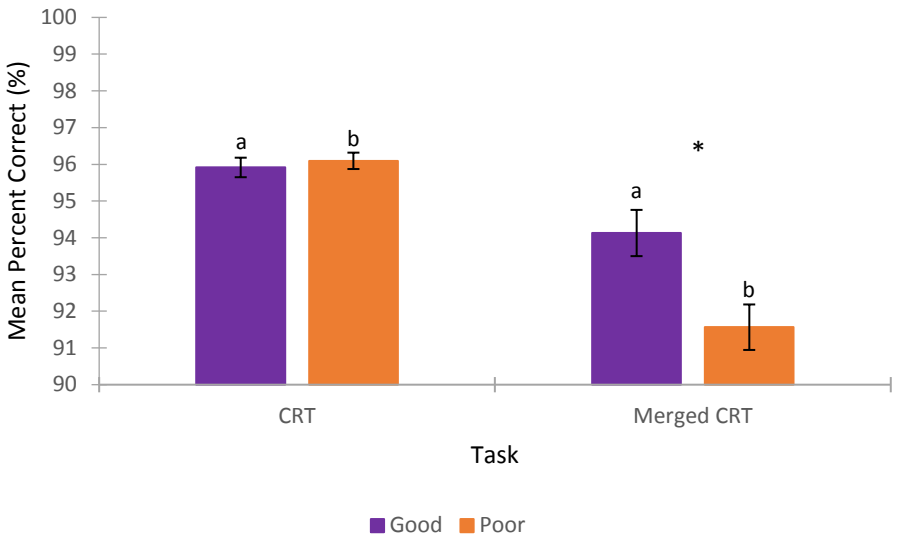


Figure 6.12: Percent Correct glucoregulatory group means for CRT and Merged tasks

Values represent estimated marginal means for good and poor regulators within each cognitive task. There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a cognitive task. Task means that share same green letter are significantly different from one another within glucoregulatory group. Error bars represent standard error of the mean. The figure shows that both glucoregulatory groups performed significantly better on the CRT task compared to Merged CRT performance. Poor glucose regulators produced a significantly lower percentage of correct responses during Merged CRT performance compared to good glucose regulators.

6.3.3.6.1.2 CRT vs. Merged Reaction Time

As expected, Task was found to be a significant predictor of Reaction Time; $F(1, 651) = 62.582$, $p < 0.001$, with participants being significantly quicker on the CRT task (M=409.53, SD=11.96) compared to Merged CRT performance (M=435.44, SD=10.19). The two-way interaction between Task and Regulator Type was also significant for Reaction Time; $F(1, 450) = 22.052$, $p < 0.001$. Post hoc tests revealed that poor glucose regulators were significantly quicker on the CRT task compared to good glucose regulators (see Figure 6.13). Interestingly, the performance drop between tasks is much greater for the poor glucoregulatory group compared to the good glucose regulators, suggesting the increased cognitive load during Merged CRT performance affects the glucoregulatory groups to a different extent (see Figure 6.13). Although, the extent of performance drop between tasks may be partly explained by the significantly quicker reaction times produced by the poor glucose regulators on the CRT task compared to good glucose regulators (see Figure 6.13). Post hoc tests also indicated an effect of Task with good and poor glucose regulators being significantly quicker on the CRT task compared to Merged CRT performance (see Figure 6.13).

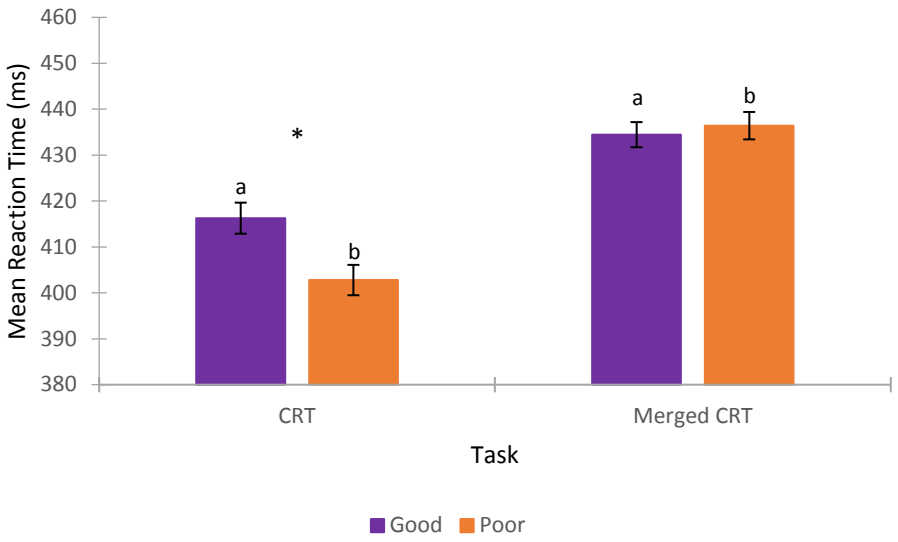


Figure 6.13: Reaction Time glucoregulatory group means for CRT and Merged tasks

Values represent estimated marginal means for good and poor regulators within each cognitive task. There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a cognitive task. Task means that share the same letter are significantly different from one another within glucoregulatory group. Error bars represent standard error of the mean. The figure indicates an effect of Task with both glucoregulatory groups being significantly quicker to respond on the CRT task compared to Merged CRT performance. The increased cognitive load of the Merged task appears to adversely affect the performance of both glucoregulatory groups, although this is observed to a greater extent in the poor glucoregulatory group. This may be partly explained by the significantly quicker reaction times of the poor glucose regulators during the CRT task compared to the good glucoregulatory group.

The three-way interaction between Condition, Task and Regulator Type was also significant for Reaction Time; $F(1, 421) = 5.577$, $p = 0.019$. Post hoc tests revealed that poor glucose regulators were significantly quicker than good glucose regulators on the CRT task during the UGP condition (see Figure 6.14). Interestingly, the greater decline in performance between tasks occurs for the poor glucoregulatory group in both conditions, which suggests that poorer glucose tolerance within the T2DM sample is associated with poorer cognitive performance under the increased cognitive load of the Merged task (see Figure 6.14). Post hoc tests also revealed a main effect of Task with good and poor glucose regulators reacting significantly slower during Merged CRT performance compared to the CRT task in both conditions (see Figure 6.14).

Figure 6.14: Reaction Time glucoregulatory group means for CRT and Merged tasks within each condition

Values represent estimated marginal means for good and poor regulators within each condition (above = FGP, below = UGP). There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between glucoregulatory groups on a cognitive task within condition. Means that share the same letter are significantly different from one another for a glucoregulatory group within condition. Error bars represent standard error of the mean. This figure shows that poor glucose regulators were significantly quicker during the CRT task than the good glucose regulators in the UGP condition. Both glucoregulatory groups were significantly slower to react during Merged CRT performance compared to the CRT task. Although, the drop in performance between tasks is greater for the poor glucoregulatory group in both conditions.

6.3.3.6.2 RVIP and Merged tasks (RVIP aspect within Merged task)

6.3.3.6.2.1 RVIP vs. Merged Percent Correct

As expected, Task was found to be a significant predictor of Percent Correct; $F(1, 465) = 140.333$, $p < 0.001$, with participants achieving a significantly higher percent of correct responses for the RVIP task (M=88.75, SD=2.37) compared to Merged RVIP performance (M=77.1, SD=5.23), indicating a performance drop between tasks. The two-way interaction between Task and Regulator Type was also significant for Percent Correct; $F(1, 376) = 4.279$, $p = 0.039$. Post hoc tests revealed that good glucose regulators were significantly more accurate during Merged RVIP performance compared to poor glucose regulators, suggesting an underlying beneficial effect of comparatively better glucose tolerance during an increased cognitive load (see Figure 6.15). Post hoc tests also revealed an effect of Task with good and poor glucose regulators being significantly more accurate on the RVIP task compared to Merged RVIP performance (see Figure 6.15).

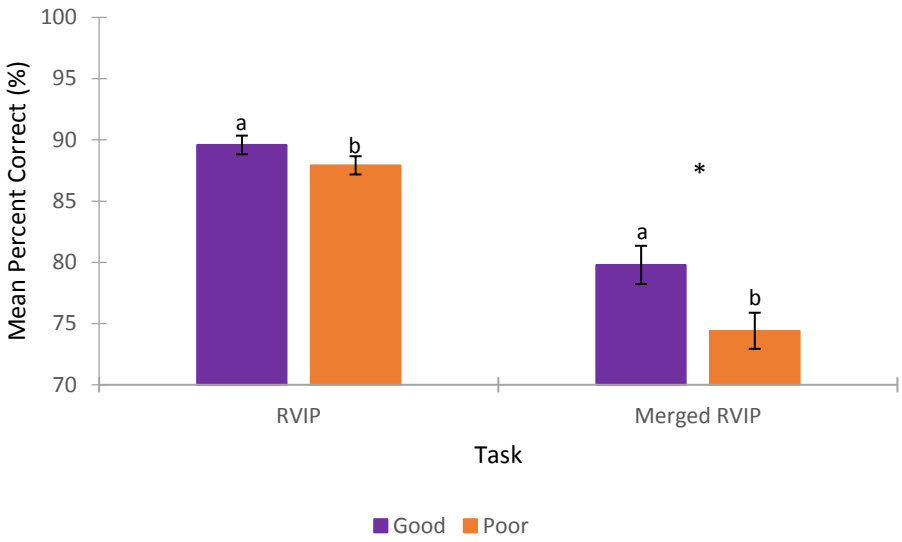


Figure 6.15: Percent Correct glucoregulatory group means for RVIP and Merged tasks

Values represent estimated marginal means for good and poor regulators within each cognitive task. There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators within a cognitive task. Task means that share the same letter are significantly different from one another with glucoregulatory group. Error bars represent standard error of the mean. The figure shows that poor glucose regulators were significantly more accurate during Merged RVIP performance compared to good glucose regulators. Both glucoregulatory groups were significantly more accurate on the RVIP task compared to Merged RVIP performance.

6.3.3.6.2.2 RVIP vs. Merged Reaction Time

As expected, Task was found to be a significant predictor of Reaction Time; $F(1, 549) = 75.198$, $p < 0.001$, with participants reacting significantly quicker on the RVIP task (M=468.11, SD=17.78) compared to Merged RVIP performance (M=510.96, SD=19.91). No significant interaction between Task and any other predictor was found, indicating that this Reaction Time difference between tasks was consistent across different levels of Condition, Time and Regulator Type.

6.3.4 Subjective Mood

6.3.4.1 Alertness

The overall mean Subjective Alertness levels in the FGP condition (M=67.79, SD=9.40) and UGP condition (M=68.46, SD=9.41) did not significantly differ, as indicated by Condition being non-significant. Subjective Alertness changed throughout the day as indicated by Time being a significant predictor, $F(4, 50) = 4.121$, $p = 0.006$. Post hoc tests revealed that Alertness levels were significantly higher during the final assessment at 17:00 compared to the second assessment after lunch at 14:00 (see Figure 6.16). Interestingly, Subjective Alertness appears to immediately rise post lunch before declining to its' lowest value across the day at 14:00,

which is followed by two increases post snack consumption throughout the afternoon (see Figure 6.16). This suggests that both the lunch and snack had a beneficial effect on Alertness levels but this effect was prolonged following the snack. This finding likely reflects the presence of a post-lunch dip.

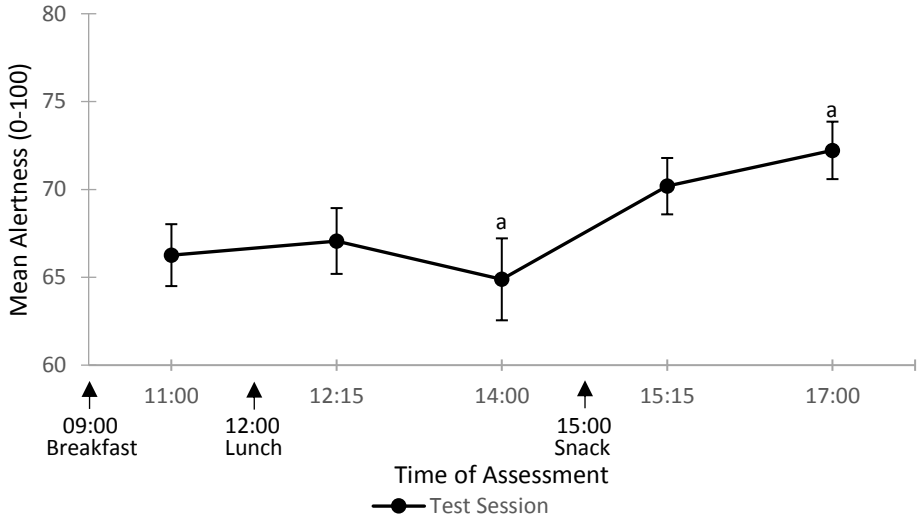


Figure 6.16: Mean Subjective Alertness for each testing session

Values represent estimated marginal means across all participants at each assessment time point across conditions. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. Baseline alertness (09:15) was included as a covariate in the linear mixed model, hence its exclusion from the figure. This figure shows that Subjective Alertness increased following lunch and snack consumption, although this effect appears prolonged following the snack. The dip in Alertness levels in the mid-afternoon and the general increases observed post snack consumption indicates the presence of a post-lunch dip.

6.3.4.2 Anxiety

The overall mean Subjective Anxiety levels in the FGP condition (M=23.06, SD=8.97) and UGP condition (M=24.69, SD=8.98) did not significantly differ, as indicated by Condition being non-significant. Neither Time or the Condition by Time interaction were found to significantly predict Subjective Anxiety, indicating that the participants felt similar levels of Subjective Anxiety between and within the FGP and UGP conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar Subjective Anxiety throughout testing.

6.3.4.3 Contentment

The overall mean Subjective Contentment levels in the FGP condition (M=79.90, SD=6.25) and UGP condition (M=79.17, SD=6.26) did not significantly differ, as indicated by Condition being non-significant. Subjective Contentment varied throughout the day as indicated by Time being a significant predictor, F(4, 50) = 4.637, p = 0.003. Post hoc tests revealed that Subjective Contentment levels were at their lowest in the morning at 11:00 and gradually increased throughout the test day with the highest levels occurring during the final assessment at 17:00 (see Figure 6.17).

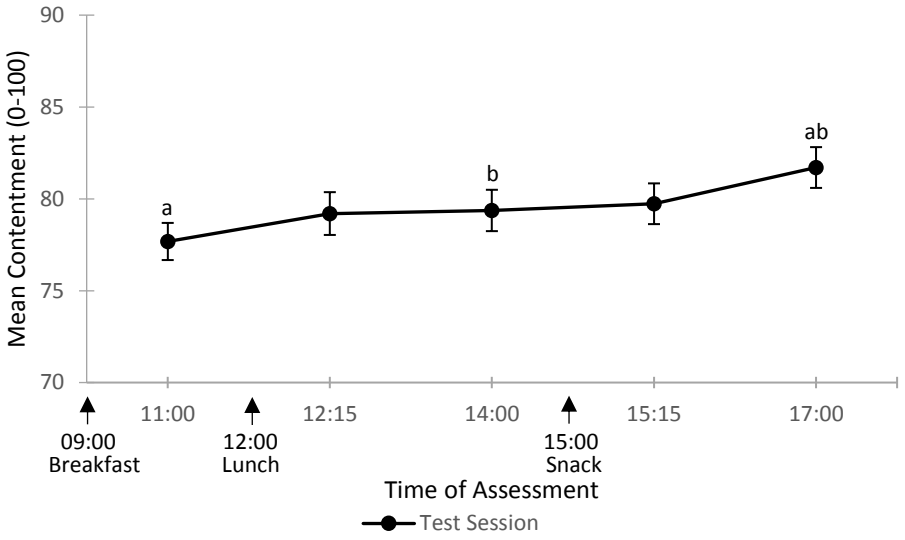


Figure 6.17: Mean Subjective Contentment for each testing session

Values represent estimated marginal means across all participants at each assessment time point across conditions. Test sessions that share the same letter are significantly different from one another. Error bars represent standard error of the mean. Black arrows represent meal consumption times. The figure shows that Subjective Contentment gradually increased throughout the test day, with the lowest levels occurring at the first assessment and the highest levels being observed at the final assessment.

Interestingly, the Time by Regulator Type interaction approached significance for Subjective Contentment, F(4, 50) = 2.091, p = 0.096. Post hoc tests revealed that good glucose regulators felt significantly more content than poor glucose regulators at 11:00 and 12:15, suggesting breakfast and lunch consumption had a greater effect on Contentment levels for the good glucoregulatory group. Although, this finding may also simply reflect naturally higher feelings of Contentment in the good glucoregulatory group. Post hoc tests also revealed that only poor glucose regulators reported significantly different Subjective Contentment levels across the day, whereas good glucose regulators were more consistently content (see Figure 6.18).

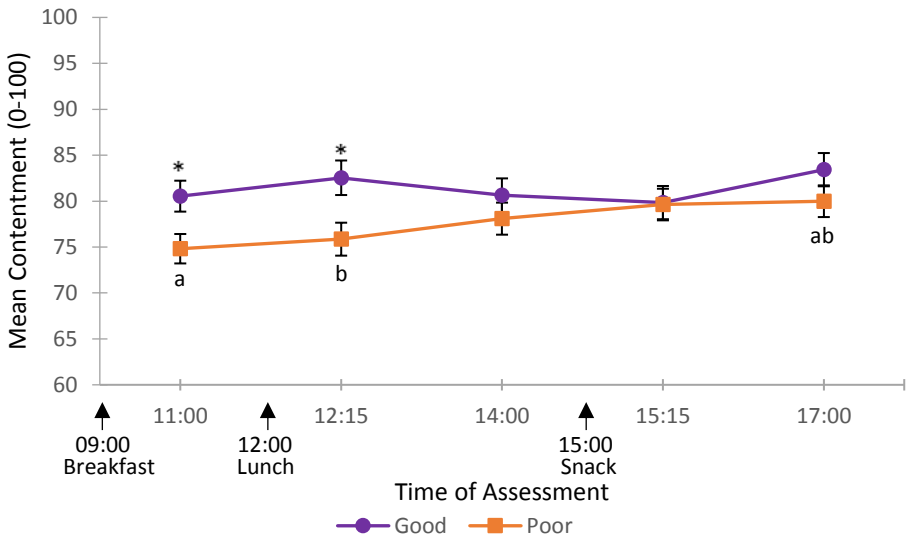


Figure 6.18: Mean Subjective Contentment for glucose regulators at each test session

Values represent estimated marginal means for good and poor glucose regulators at each testing session across conditions. There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between glucoregulatory groups at an assessment time point. Test sessions that share the same letter are significantly different from one another with glucoregulatory group. Error bars represent standard error of the mean. The figure indicates that good glucose regulators were significantly more content during the earlier assessments compared to the poor glucose regulators. The poor glucoregulatory group was significantly more content during the final assessment compared to the earlier assessments, whilst good glucose regulators did not significantly differ throughout the day.

6.3.5 Subjective Hunger, Fullness and Sleepiness (HFS)

6.3.5.1 Hunger

The overall mean Subjective Hunger levels in the FGP condition (M=24.73, SD=16.73) and UGP condition (M=27.26, SD=16.92) did not significantly differ, as indicated by Condition being non-significant. As expected, Subjective Hunger changed throughout the day as indicated by Time being a significant predictor, $F(4, 50) = 3.016$, $p = 0.026$. Furthermore, the Condition by Time interaction was also a significant predictor of Subjective Hunger, $F(4, 50) = 2.919$, $p = 0.03$. Post hoc tests revealed that Subjective Hunger was highest in the morning at 11:00 for both conditions, with the lowest levels occurring at 12:15 in the FGP condition and at 15:15 for the UGP condition. Interestingly, snack consumption was followed by a decrease in Subjective Hunger in the UGP condition as expected but was followed by an increase of hunger levels in the FGP condition, indicating participants found the FGP snack to be less satiating than the UGP snack (see Figure 6.19).

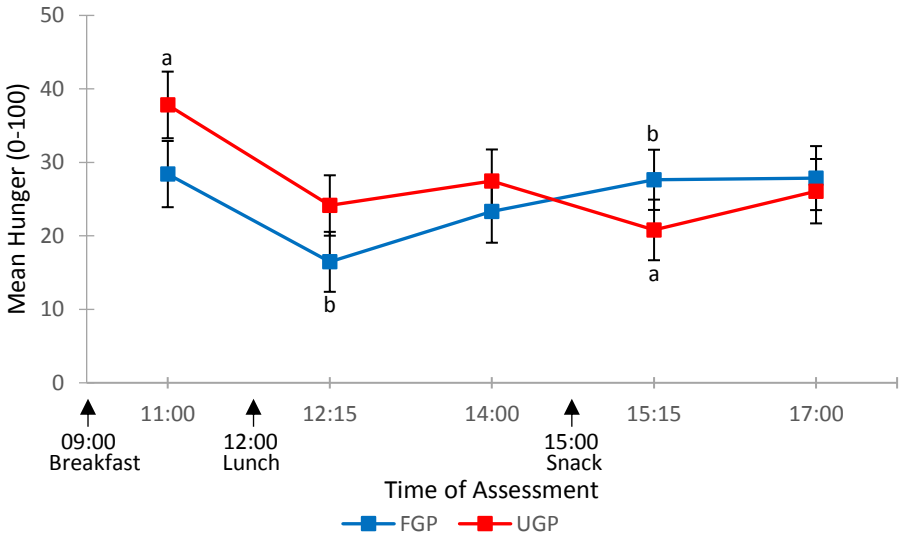


Figure 6.19: The interaction between Condition and Time for Subjective Hunger

Data points represent estimated marginal means across all participants at each time point. Test sessions that share the same letter are significantly different from one another within condition. Black arrows represent meal consumption times. Error bars represent standard error of the mean. This figure shows that Subjective Hunger levels significantly varied throughout the day in both conditions. Divergent patterns of Subjective Hunger were observed between conditions following snack consumption, which suggests that the UGP snack had a greater satiating effect than the FGP snack.

Good glucose regulators (M=43.18, SD=23.06) were significantly hungrier than poor glucose regulators (M=20.35, SD=19.83, see figure 6.20) in the UGP condition, as indicated by post hoc tests following the Condition by Regulator Type interaction trending towards significance; $F(1, 46) = 2.97$, $p = 0.092$. The three-way interaction between Condition, Time and Regulator Type also approached significance; $F(4, 50) = 2.309$, $p = 0.071$. As shown in Figure 6.20, poor glucose regulators reported significantly lower levels of Subjective Hunger at 12:15 than at 15:15 and 17:00 in the FGP condition. The poor glucoregulatory group were also significantly hungrier at 11:00 than at 15:15 and 17:00 in the UGP condition. Interestingly, poor glucose regulators reported increasing levels of hunger throughout the FGP condition after an initial drop at 12:15, but the opposite pattern is observed for the same glucoregulatory group throughout the UGP condition (see Figure 6.20). This finding suggests that the FGP and UGP meals had differential effects on Subjective Hunger for the poor glucoregulatory group. Post hoc tests also revealed that poor glucose regulators felt significantly less hunger than good glucose regulators at 14:00, 15:15 and 17:00 in the UGP condition, whereas no significant differences between glucoregulatory groups were observed in the FGP condition (see Figure 6.20).

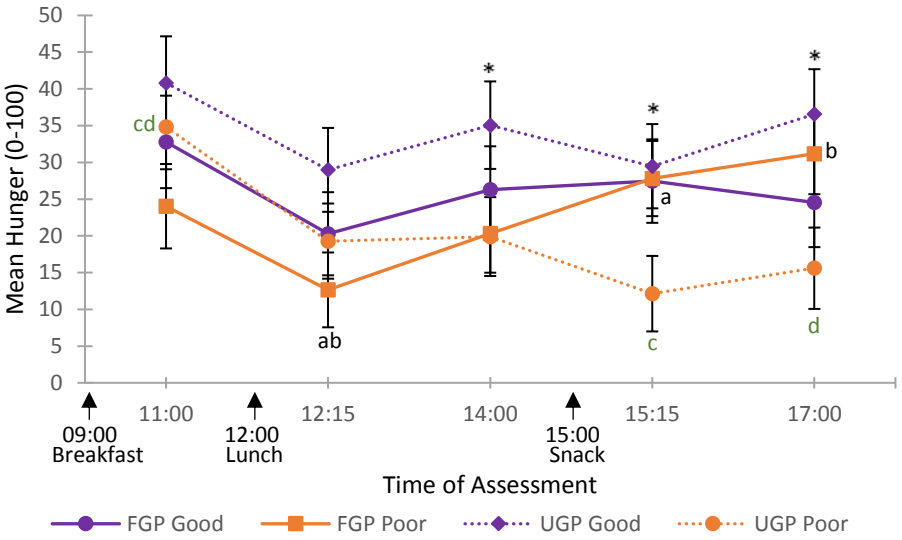


Figure 6.20: Subjective Hunger levels for good and poor glucose regulators within each condition

Values represent estimated marginal means for good and poor regulators at assessment time points within each condition (above = FGP, below = UGP). There were 12 good and 13 poor glucose regulators. An asterisk (*) indicates a significant difference between good and poor regulators at an assessment time point in the UGP condition only. Test sessions that share the same black letter are significantly different from one another for the poor glucoregulatory group within the FGP condition only. Test sessions that share the same green letter are significantly different from one another for the poor glucoregulatory group within the UGP condition only. Error bars represent standard error of the mean. The figure indicates that poor glucose regulators were significantly hungrier than good glucose regulators across the afternoon UGP assessments compared to poor glucose regulators. Divergent patterns of Subjective Hunger between conditions are observed for the poor glucoregulatory group with levels increasing after the 12:15 assessment in the FGP condition, whilst decreasing after the same assessment in the UGP condition.

6.3.5.2 Fullness

The overall mean Subjective Fullness levels in the FGP condition (M=66.91, SD=11.92) and UGP condition (M=63.46, SD=11.92) did not significantly differ, as indicated by Condition being non-significant. Subjective Fullness significantly varied throughout the day as indicated by Time being a significant predictor, $F(4, 50) = 6.497$, $p < 0.001$. As expected, Subjective Fullness increased following meal consumption, with the greatest increase occurring after the consumption of the lunch meal. The snack led to a smaller increase in Subjective Fullness, reflecting its' lower energy and macronutrient content compared to the lunch meal (see Figure 6.21).

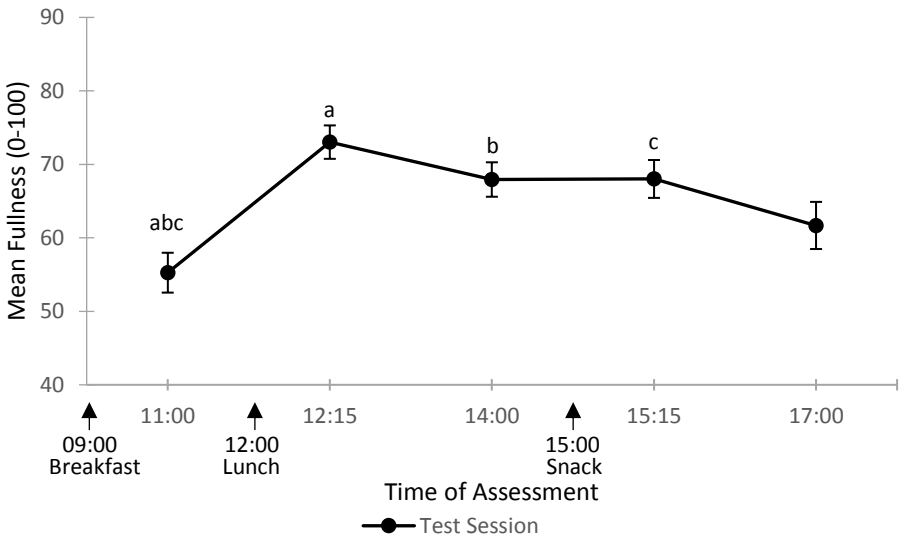


Figure 6.21: Mean Subjective Fullness for each testing session

Values represent estimated marginal means across all participants at each time of assessment. Test sessions that share the same letter are significantly different from one another. Black arrows represent meal consumption times. Error bars represent standard error of the mean. The figure shows that meal consumption led to an increase in Subjective Fullness, with the largest increase being observed post lunch.

Considering glucose regulation, glucoregulatory groups reported significantly different overall levels of Subjective Fullness as indicated by Regulator Type being significant, $F(1, 56) = 5.725$, $p = 0.2$. Post hoc tests revealed that good glucose regulators ($M=59.84$, $SD=14.33$) reported significantly lower Subjective Fullness compared to poor glucose regulators ($M=70.54$, $SD=13.61$). Furthermore, the Condition by Regulator Type interaction approached significance, $F(1, 48) = 2.863$, $p = 0.097$. Post hoc tests revealed that poor glucose regulators were significantly fuller than good glucose regulators in the UGP condition, whilst glucoregulatory groups did not significantly differ in the FGP condition (see Figure 6.22). Neither glucoregulatory group reported significantly different levels of Subjective Fullness between conditions.

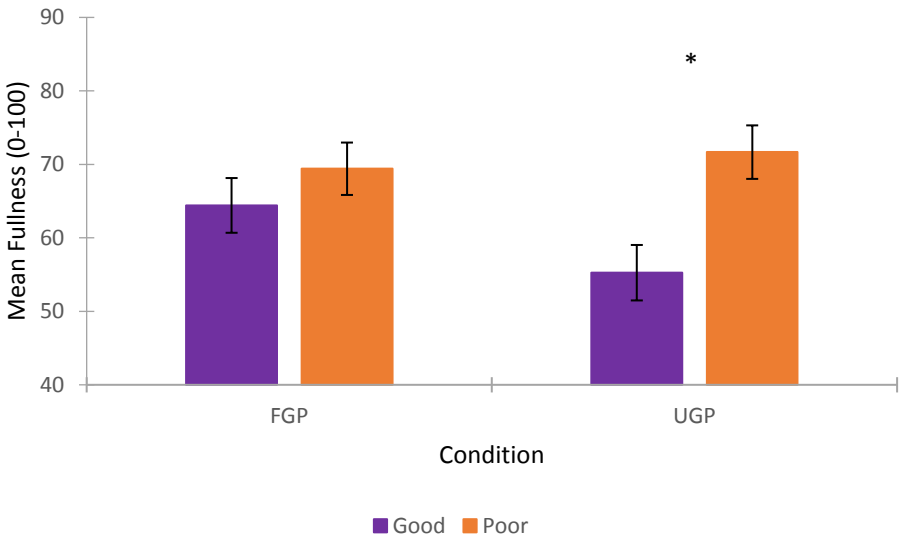


Figure 6.22: Subjective Fullness condition means for good and poor glucose regulators

Values represent estimated marginal means for good and poor regulators within each condition. There were 12 good and 12 poor glucose regulators. Data points with an asterisk (*) indicate a significant difference between glucoregulatory groups within a condition. Error bars represent standard error of the mean. The figure indicates that poor glucose regulators were significantly fuller in the UGP condition compared to good glucose regulators, whilst no significant differences between glucoregulatory groups were observed during the FGP condition.

6.3.5.3 Sleepiness

The overall mean Subjective Sleepiness levels in the FGP condition (M=39.64, SD=12.80) and UGP condition (M=41.11, SD=12.82) did not significantly differ, as indicated by Condition being non-significant. The Condition by Time interaction approached significance for Subjective Sleepiness, $F(4, 50) = 2.521$, $p = 0.053$. However, Bonferroni corrected post hoc tests revealed no significant differences between conditions at any assessment time point. Time did not significantly predict Subjective Sleepiness, indicating that the participants felt similar levels of Subjective Sleepiness throughout the day across conditions. Regulator Type was also non-significant, indicating that glucoregulatory groups reported similar Subjective Sleepiness throughout testing.

6.3.6 Summary of findings

The collective findings from the analysis of glycaemic, cognitive performance and subjective mood measures can be found in Table 6.2.

Table 6.2: Summary of glycaemic, cognitive performance and subjective mood findings.

Measure	Benefits for FGP	Benefits for UGP	Benefits for good glucose regulation	Benefits for poor glucose regulation
Glycaemic Response	A lower glycaemic response after lunch and snack consumption.	None observed.	A lower glycaemic response after breakfast, lunch and snack consumption.	None observed.
Cognitive Performance	Higher RVIP accuracy immediately before lunch. Breakfast was associated with sustained Global Accuracy, Reaction Time and Performance across the morning.	Higher CRT accuracy immediately after lunch. Gradual Global Reaction Time improvements followed the lunch meal, continuing post snack, suggesting a beneficial second meal cognitive effect of lunch.	Higher Merged CRT accuracy. Higher Merged RVIP accuracy.	Faster overall CRT reaction times.
Subjective Mood	None observed.	None observed.	Consistently higher levels of subjective contentment.	None observed.
Subjective ratings of Hunger, Fullness and Sleepiness	None observed.	None observed.	None observed.	Less hungry throughout the afternoon in the UGP. Fuller overall in the UGP.

6.4 Discussion

The primary aim of this study was to investigate whether there were any significant differences in cognitive performance between a favourable glucose profile (FGP) and an unfavourable glucose profile (UGP) in a T2DM population. The second aim was to investigate whether glucose tolerance status within a T2DM population predicted cognitive performance. This study also aimed to investigate whether any significant differences in subjective mood ratings were found between the FGP and UGP conditions in a T2DM population. The final aim was to investigate whether glucose tolerance status within a T2DM population predicted subjective mood ratings.

6.4.1 The Glycaemic Response

It was important to consider the glycaemic response profiles produced by the FGP and UGP conditions in the current T2DM sample. To summarise, the glycaemic response profiles were as expected with a significantly higher glycaemic response in the UGP condition. Interestingly, the largest differences in glycaemic response between conditions occurred following lunch consumption. This finding also occurred in the previous two studies (Chapter 4 & 5) and is likely a reflection of the lunch meals having the largest difference in GI values, which would be expected to produce the most divergent glycaemic responses according to the GI concept (Jenkins et al., 1981). However, the glycaemic response to the breakfast meal was remarkably similar for both conditions. For clarity, the glycaemic response following the UGP breakfast is a high glycaemic response as expected, but the glycaemic response following the FGP breakfast appears much higher than expected. Considering that previous nutritional consumption, the evening prior to testing was controlled for and the crossover research design of the study it is plausible that the inclusion of apple juice is likely to have augmented the FGP breakfast glycaemic response. For example, the inclusion of apple juice may lead to a higher glycaemic response due to (i) it's absorption rate and (ii) it's fructose content. According to the dietary fibre hypothesis, it would be expected that a liquid, containing little to no fibre, will be more quickly absorbed by the gut compared to a high fibre food (Burkitt & Trowell, 1977). As apple juice is a liquid and contains little fibre, it would be absorbed quickly by the gut and could lead to the rapid increase in glucose concentration seen the FGP condition. Furthermore, research has found that fructose may increase glucokinase activity, which could lead to increased hepatic glucose uptake and reduced hepatic glucose output (Le & Tappy, 2006; Wolever et al., 2009; Wolf et al., 2002). The result of this would be a reduction in postprandial glycaemia, which may explain the sharp drop in glucose levels seen in the FGP condition following breakfast consumption (see Section 6.3.2).

The comparison of glucoregulatory groups yielded a number of interesting findings. Firstly, the data confirms and supports the diagnosis of T2DM with all assessment points of both glycaemic response profiles being above 7mmol/L, with a fasting glucose level equal or above 7mmol/L for both glucoregulatory groups (which is consistent with T2DM diagnostic criteria; WHO, 2016). Secondly, the poor glucoregulatory group produced a significantly higher glycaemic response from 90 minutes post breakfast until the end of the day as would be

expected from a group with comparatively poorer glucose tolerance. Finally, the good glucose regulators returned to baseline within 180-195 minutes after every meal whereas the poor glucose regulators did not return to baseline at any point throughout testing. Although, the poor glucoregulatory group did return to pre-lunch glucose concentrations approximately 165-180 minutes post lunch consumption. According to Sadler (2011), a healthy individual with NGT and good insulin sensitivity can be expected to return to baseline glucose concentrations within 120-180 minutes after meal consumption. Taken together, these findings support the diagnosis of T2DM and indicate that poor glucose regulators within the T2DM sample are producing a higher glycaemic response compared to good glucose regulators, which results in them experiencing prolonged states of hyperglycaemia without returning to baseline levels at any point throughout the day (see Section 6.3.2). It is likely that these differences reflect the progression of insulin resistance and its' deleterious effects on insulin-mediated glucose uptake rates of cellular tissue such as muscle and fat cells (James et al., 1988; Kahn & Pessin, 2002; Watson et al., 2004). However, it is important to note that insulin was not measured in the present study meaning that differences in insulin function between glucoregulatory groups cannot be confirmed by the present data.

6.4.2 Cognitive Performance

Interestingly, there appears to be a large drop in all three global measures throughout the morning sessions in the UGP condition, whereas performance is generally maintained in the FGP condition. This suggests that the LGI breakfast in the FGP condition was more beneficial to cognitive performance across the morning compared to the HGI breakfast in the UGP condition, which supports the findings of previous research (Benton et al., 2003; Ingwersen et al., 2007; Mahoney et al., 2005; Micha et al., 2010, 2011). This may be explained by a steadier glucose release of the LGI breakfast in the FGP condition (Jenkins et al., 1981), and a subsequent steadier supply of glucose to the brain across the morning. Indeed, differential effects of the breakfast meals on the availability of glucose to the brain across the morning likely explains why the only significant difference between conditions occurred immediately before lunch with participants producing a significantly lower Global Performance score in the UGP condition compared to the FGP condition. For Global Reaction Time, general improvements are observed at each subsequent assessment post lunch in the UGP condition, which continue following snack consumption. This finding suggests that the UGP lunch may be providing a beneficial second meal cognitive effect, following the UGP snack (Lamport et al., 2011). For Global Accuracy and Global Performance, there appears to be a dip during the mid-afternoon in both conditions, whilst general improvements in all three global measures were observed following snack consumption. These improvements appeared more immediately following the FGP snack compared to the UGP snack, although it is unclear why this is the case. These findings suggest that the provision of energy, via snack consumption, improved cognitive performance. These findings may also reflect the natural circadian rhythm of the human body, where a mid-afternoon dip in performance occurs due to an increase in human sleep propensity, which is later overwhelmed by a circadian arousal process that becomes maximal in the evening (Broughton, 1998; Campbell, 1984; Carrier & Monk, 2000; Carskadon & Dement, 1992; Lavie, 1986; Monk, 2005; Richardson et al., 1982). Finally, significant

differences between assessments were only observed in the UGP condition for all three global measures, which suggests that the FGP condition was generally more beneficial to cognition as evidenced by more stable performance.

The analysis of individual task data produced mixed findings, with beneficial cognitive effects of both conditions being observed dependent on the task. Specifically, participants were significantly more accurate on the CRT task immediately after lunch in the UGP condition compared to the FGP condition. Whereas, participants were significantly more accurate on the RVIP task immediately before lunch in the FGP condition compared to the UGP condition. These findings indicate that the cognitive domains of psychomotor function and sustained attention benefited from different conditions. In the present study, sustained attention appears to benefit from LGI meal consumption, which is in line with previous research (see Chapter 2). Whereas, psychomotor function seems to benefit from HGI meal consumption, which is in contrast to Papanikolaou et al. (2006) who found greater improvements in psychomotor function during a LGI condition. However, a review of the current literature (see Chapter 2) indicated that this was the only previously significant finding when psychomotor function was investigated, which suggests further research into this cognitive domain is required before a LGI or HGI condition can be generally considered as beneficial. Given that neural activity has been shown to determine glucose uptake rates in the brain, with higher cognitive load being associated with reduced brain extracellular glucose concentrations (Chen et al., 1993; McNay et al., 2000), it is likely that the performance differences observed here may be explained by the availability of glucose in the brain during the time of cognitive assessment. Considering that the FGP meals had a comparatively lower GI than the UGP meals, a slower glucose release into the bloodstream would be expected (Jenkins et al., 1981), which is supported by the glycaemic data (see Section 6.3.2). This would result in a steadier supply of glucose to the brain compared to the UGP breakfast. Given that the difference in sustained attention performance between conditions occurs three hours post breakfast consumption, it is possible that the steadier supply of glucose to the brain from the FGP breakfast leads to higher brain extracellular glucose availability resulting in better performance during neural activity. It is important to note that the glycaemic responses are remarkably similar post breakfast for both conditions, which may suggest that glucose delivery rates to the brain do not differ at a glance. However, the inclusion of apple juice and the effects of its' fructose content could be augmenting the glycaemic response produced by the FGP breakfast, which still contained more fibrous food than the UGP breakfast and may still be being absorbed at a slower rate (Burkitt & Trowell, 1977). Using the same logic, it is likely that a quicker glucose release from the UGP lunch leads to a higher availability of glucose to the brain during the first post-lunch assessment. This offers a plausible explanation for the better psychomotor function performance during the first post lunch assessment in the UGP condition compared to the FGP condition.

The Merged task did not produce any significant differences between conditions, assessment times or glucoregulatory groups. A closer look at the data revealed that the lowest mean percentage of correct responses during an individual assessment time in either condition was 75% across participants. This suggests that the task may have been too easy for participants, resulting in high performance throughout testing. For the LM task, there was evidence of a practise effect as demonstrated by increased accuracy at each subsequent session. This is

similar to the finding reported in Study 2 where subsequent improvements in LM reaction time were observed across the day (see Chapter 5). The current finding supports the previous suggestion that participants found the LM task more difficult than the other cognitive tasks and required repeated exposure to the task in order to improve proficiency (Bell et al., 2018). Interestingly, good glucose regulators did not significantly differ in LM reaction time throughout the day in either condition, whereas poor glucose regulators did in both conditions (see Section 6.3.3.4.2). In the FGP condition, poor glucose regulators were significantly slower in the morning compared to immediately after lunch. Given that reaction times generally improved from the morning into the first assessment post lunch for the poor glucoregulatory group in the FGP condition, this finding could reflect a second meal cognitive effect from the FGP breakfast occurring after lunch consumption. Previous research has indicated that the GI of one meal can have beneficial effects on cognitive performance after consumption of the subsequent meal (Lamport et al., 2011). In the UGP condition, poor glucose regulators were significantly slower immediately after lunch compared to the final afternoon assessment. However, general improvements in reaction time were observed throughout the afternoon for the poor glucoregulatory group post UGP lunch consumption, which continue following snack consumption. This finding suggests that a beneficial second meal cognitive effect from the UGP lunch may also be occurring after snack consumption (Lamport et al., 2011). The possibility of a second meal cognitive effect occurring throughout the day here is an important finding as previous research has only reported evidence of this effect after manipulating an evening meal and providing participants with a standardised HGI breakfast the following morning (Lamport et al., 2011). However, to confirm the presence of a second meal cognitive effect in the present study, a standardised meal would have to be consumed after the breakfast and lunch meals, followed by cognitive assessments. If better performance was observed in the FGP breakfast condition following a standardised lunch, or in the UGP lunch condition following a standardised snack, then the presence of a second meal cognitive effect would be confirmed. Finally, an interesting pattern emerged whereby good glucose regulators were generally more accurate but slower to react during the FGP condition compared to the UGP condition, whilst the reverse pattern was observed for the poor glucose regulators. This suggests that both glucoregulatory groups are committing an accuracy-reaction time trade-off, where one component is sacrificed for improvements in the other (Bogacz et al., 2010; Fitts, 1966; Reed, 1973; Wickelgren, 1977; Wood & Jennings, 1976).

As with Study 2 (Chapter 5), the impact of task difficulty was considered. The results indicated that participants were significantly more accurate on the CRT and RVIP tasks compared to their respective performance aspects within the Merged task. Interestingly, participants were also significantly faster on both the CRT and RVIP task compared to Merged CRT and Merged RVIP performance. Taken together, these findings suggest that the increased cognitive load of the Merged task resulted in poorer cognitive performance. Interestingly, the poor glucose regulatory group were significantly less accurate during both Merged CRT and Merged RVIP performance compared to the good glucoregulatory group, whilst no significant differences in reaction time were observed between groups. This finding is similar to those reported in Study 2 (see Chapter 5) and suggests that glucose tolerance may have a moderating role in the relationship between glycaemic response and cognitive performance, which becomes more apparent under an increased cognitive load. Previous research has indicated poorer glucose

tolerance is associated with the dysfunction of a number of mechanisms which can adversely affect cognitive performance (see Chapter 2). For example, poorer glucose tolerance has been associated with BBB endothelium dysfunction (Baron, 1996; Benatti et al., 2016; Brownlee, 2001; Cohen, 1993; Huber et al., 2008; Su et al., 2008), which can adversely affect the transport of glucose into the brain. During a higher cognitive load such as the Merged task here, it would be expected that the brain's demand for glucose is greater than performance on an "easier" task (Chen et al., 1993; McNay et al., 2000). Therefore, an inability to supply the brain with enough glucose, due to dysfunction of the BBB endothelium, may become more apparent under an increased cognitive load. In context of the present study, it is plausible that the poor glucoregulatory group have suffered greater injury to the structural integrity of the BBB compared to the good glucoregulatory group, with the adverse cognitive effects of this only becoming apparent during the increased cognitive load of the Merged task. Finally, poor glucose regulators were quicker to react on the CRT task compared to good glucose regulators in both conditions, although this difference was only significant in the UGP condition. Considering that poor glucose regulators generally reacted quicker in both conditions but accuracy between groups was remarkably similar, this finding suggests that the poor glucoregulatory group simply found the CRT task easier to perform than the good glucoregulatory group.

To summarise, analysis of the cognitive data returned mixed results, with the cognitive domains of psychomotor function and attention benefitting from UGP lunch and FGP breakfast consumption respectively. The examination of the three global cognitive measures indicated that a LGI breakfast is associated with sustained cognitive performance, whereas a HGI breakfast appears detrimental to cognitive performance, across the morning. This finding supports those reported by the previous research reviewed here (see Chapter 2). A plausible explanation for this is the differential effects that these meals have on rate of glucose release into the bloodstream and the subsequent availability of glucose to the brain at the time of assessment. Significant differences between glucoregulatory groups were not observed on the simpler tasks, which may reflect the overall T2DM status shared between participants. However, comparisons between tasks revealed that poor glucose regulators performed significantly worse than good glucose regulators under the increased cognitive load of the Merged task. This finding suggests that the dysfunction of underlying mechanisms that associate poorer glucose tolerance with cognitive impairment become more apparent during an increased cognitive load, where the brains' demand for glucose is higher. The LM task, a measure of executive function, appeared to display practise effects with participants becoming more accurate with repeated exposure to the task (Bell et al., 2018). Although, a general pattern emerged whereby good glucose regulators were generally more accurate but slower to react in the FGP condition, whilst the reverse pattern was observed for the poor glucoregulatory group. This finding suggests an accuracy-reaction time trade-off may be occurring in both glucoregulatory groups during each condition (Bogacz et al., 2010; Fitts, 1966; Reed, 1973; Wickelgren, 1977; Wood & Jennings, 1976). Finally, there was some evidence for the presence of a second meal cognitive effect occurring in both conditions, although further testing would be required to confirm this.

6.4.3 Subjective Mood

The subjective mood data analysis returned minimal findings, with significant differences being largely limited to between assessments. To summarise, there were no significant differences between conditions for any of the subjective mood factors; alertness, anxiety and contentment. This is likely due to (i) a consistent testing environment that avoids social influence (Bowling, 2005), (ii) the energy and macronutrient matching between meals, and (iii) the overall T2DM glucose tolerance status of the sample. For example, by matching protein and fat contents, their augmenting effect on the insulin response (Gunnarsson et al., 2006; Jenkins et al., 1981) and the associated elevation of neurotransmitters such as norepinephrine, which has been to shown to increase alertness and anxiety, is avoided (Aston-Jones, 1981; Oken, Salinksy & Elsas, 2006; Southwick et al., 1999; Trulson & Himmel, 1985; Wise & Stein, 1969). Whilst GI/GL variation between the meals could lead to divergent insulin responses (Jenkins et al., 1981) and norepinephrine levels, it is plausible that the level of insulin secreted in the T2DM participants does not vary enough between meals or throughout the day to produce significantly different ratings of subjective mood, even in the presence of divergent glycaemic profiles. This is likely considering that all participants had T2DM, which has been associated with reduced insulin secretion (ADA, 2013). Given that the present study did match test meals for energy and macronutrients and found no significant mood differences between conditions, it is possible that mood differences reported by previous research have occurred due to energy and macronutrient mismatching between test meals (see Chapter 2). For example, Nabb & Benton (2006) provided participants with one of eight meals varying in CHO and fibre content and found that higher amounts of CHO were associated with higher levels of tiredness. Therefore, it is vital that future research carefully matches the energy and macronutrient content between conditions to avoid potential confounding effects on subjective mood. Interestingly, subjective alertness and contentment share the same trend in the present study, with increases being seen throughout the day. This suggests that the provision of energy, through meal consumption, may be having positive effects on subjective mood. It is also plausible that alertness and contentment are linked, with participants simply reporting increasing levels of contentment as they are feeling more alert throughout the day. The lowest levels of subjective alertness were reported in the afternoon after lunch consumption, which suggests the presence of a post-lunch dip (Monk, 2005). Finally, subjective contentment significantly differed between glucoregulatory groups in the earlier assessments but poor glucose regulators generally reported lower contentment throughout the day compared with good glucose regulators although it is unclear why this is the case.

6.4.4 Subjective ratings of Hunger, Fullness and Sleepiness

The analysis of subjective hunger, fullness and sleepiness returned a number of significant findings. As expected, overall subjective fullness increased after each meal. Although, poor glucose regulators felt significantly fuller on average than good glucose regulators in the UGP condition. For subjective hunger, the good glucoregulatory group followed an expected pattern in both conditions, with meal consumption reducing subjective hunger ratings. Whilst

poor glucose regulators also followed this pattern in the UGP condition, the reductions in subjective hunger appear larger than those observed in the good glucose regulators. This led to poor glucose regulators being significantly less hungry during the afternoon sessions compared with good glucose regulators in the UGP condition. It is plausible that reduced insulin sensitivity in the poor glucoregulatory group may result in circulating insulin levels remaining elevated for a longer duration compared to the good glucoregulatory group (ADA, 2013). As elevated insulin levels have been associated with reduced secretion of the hunger-stimulating hormone ghrelin (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014), this could result in the lower subjective hunger ratings reported by the poor glucoregulatory group in the UGP afternoon sessions. This would also explain why the poor glucoregulatory group reported a significantly higher overall subjective fullness rating in the UGP condition compared to good glucose regulators. In the FGP condition, the poor glucose regulators reported increasing levels of subjective hunger throughout the afternoon, even after snack consumption. It is possible that the combination of lower insulin responses produced by the LGI meals in the FGP condition (Jenkins et al., 1981) and reduced insulin secretion (ADA, 2013) may be leading to increased ghrelin production in the poor glucoregulatory group during the FGP condition. This offers a plausible explanation for the increasing subjective hunger levels reported by the poor glucose regulators during the FGP afternoon sessions. Although, it should be noted that whilst the mechanistic role of insulin in ghrelin production provides a logical explanation for these findings, it cannot be confirmed by the present data as insulin was not measured. Finally, no significant differences between conditions or glucoregulatory groups were observed for subjective sleepiness, which mirrors the findings for subjective alertness (see Section 6.4.3). Although, general subjective alertness significantly differed throughout the day, whereas general subjective sleepiness did not, which may simply reflect different interpretations of the measures by participants during self-reporting. For example, participants may have viewed the subjective alertness measure as the reporting of energetic feeling but the subjective sleepiness measure as an inclination to sleep.

6.5 Conclusions

In conclusion, this study identified a number of significant findings concerning the glycaemic response, cognitive performance and mood. Firstly, the two meal profiles were a success with the UGP condition producing a significantly higher glycaemic response compared to the FGP condition, which is in line with previous research showing higher glycaemic responses to HGI meals (Jenkins et al., 1981). As expected, poor glucose regulators produced a significantly higher glycaemic response compared to good glucose regulators. The global cognition measures data indicated that the FGP breakfast was associated with better cognitive performance across the morning compared to the UGP breakfast, which supports the findings of previous research (Benton et al., 2003; Ingwersen et al., 2007; Mahoney et al., 2005; Micha et al., 2010, 2011). It was also apparent that the FGP condition was more beneficial to cognition than the UGP condition as evidenced by more stable performance across the day. Analysis of LM task Reaction Time and Global Cognitive Reaction Time suggested the presence of a beneficial second meal cognitive effect from the UGP lunch, which is observed after snack consumption. A beneficial second meal cognitive effect from the FGP breakfast also appeared

to be present for LM task Reaction Time following lunch consumption. The finding of a potential second meal cognitive effect occurring during the day here is important as the current literature has only reported a second meal cognitive effect of an evening meal after a standardised breakfast the following morning (Lamport et al., 2011). However, further testing of cognitive performance and these meals, with the addition of a standardised subsequent meal, would be required to confirm the presence of a second meal cognitive effect. Results also indicated that psychomotor function and sustained attention followed an opposing pattern, with beneficial effects of the UGP lunch on psychomotor function being observed, whilst sustained attention performance appeared to benefit from the FGP breakfast. Improved performance in these areas at an individual assessment time can be attributed to higher brain extracellular glucose concentrations (Abi-Sabb et al., 2002; Gruetter et al., 1996; Harada et al., 1993; Jacob et al., 2002; Messier, 2004) brought about by varied gut absorption rates of the test meals (Burkitt & Trowell, 1977; Jenkins et al., 1981). Significant differences in cognitive performance between glucoregulatory groups were limited to Merged CRT and RVIP performance where poor glucose regulators performed significantly worse than good glucose regulators. This finding suggests injury to underlying mechanisms associating poorer glucose tolerance and cognitive impairment become more apparent under an increased cognitive load (see Chapter 2).

Significant findings for subjective mood ratings were largely limited to differences between assessments, with alertness and contentment increasing throughout the day. This finding suggests that the two mood factors may be linked, with participants reporting higher contentment as they feel more alert. Subjective contentment significantly differed between glucoregulatory groups in the earlier assessments but poor glucose regulators generally reported lower contentment throughout the day compared with good glucose regulators although it is unclear why this is the case. Finally, significant differences between glucoregulatory groups were observed for both subjective hunger and fullness. Specifically, poor glucose regulators felt significantly less hunger, and fuller, during the UGP condition compared to the good glucose regulators. This may be explained by reduced insulin sensitivity in the poor glucose regulators leading to longer durations of the elevated insulin levels, which has been associated with reduced ghrelin production and subsequently less hunger (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014; Dickson et al., 2011; Inui et al., 2004; Meier & Gressner, 2004; Sakata & Sakai, 2010; Schwartz et al., 2000). The next question of interest is whether significant differences in glycaemic response, cognitive performance and subjective mood occur when a healthy and T2DM sample are compared.

Chapter 7

General Discussion

The relationship between the glycaemic response and cognitive performance has been previously investigated through dietary interventions (see Chapter 2). Whilst some studies have implemented the GI/GL concept to manipulate the glycaemic response produced by test conditions, the majority of cognitive research has only investigated a single meal, particularly breakfast. This thesis extended the previous single meal testing paradigm to a multiple meal investigation across the day and utilised the GI concept to design two glycaemic meal conditions, each consisting of three LGI or HGI meals (Jenkins et al., 1981). By doing so, this thesis aimed to investigate the relationship between the glycaemic response, cognitive performance and subjective mood over the course of three consecutive meals. The studies (Chapter 4-6) in this thesis implemented the use of a glucose composite score to separate comparatively good and poor glucose regulators within each sample that was examined. A general summary of the key findings from the research conducted throughout this thesis can be found below (see Section 8.1).

7.1 Summary of thesis findings

7.1.1 Glycaemic Response

As aforementioned, for the purpose of this thesis the GI concept was implemented in order to design two distinctly different meal profiles, each consisting of a breakfast, lunch and afternoon snack (Jenkins et al., 1981). For clarity, the FGP condition consisted of entirely LGI meals, whereas the UGP condition consisted of solely HGI meals. Overall, the glycaemic response profiles produced by both glycaemic conditions largely displayed an expected pattern throughout this thesis, with the UGP condition producing a significantly higher glycaemic response compared to the FGP condition for the majority of the day. The largest significant differences between conditions were observed in the mid-afternoon following lunch consumption in all studies, which was expected as the lunch meals also had the largest difference in GI values. Taken together, these findings support the clinical utility of the GI concept and the show the expected glycaemic differences following either LGI or HGI meal consumption (Wolever & Jenkins, 1986; Wolever et al., 1991). However, the glycaemic response following the FGP breakfast in Chapter 4 is noticeably larger than the glycaemic response following the same meal in Chapter 5. It is likely that this inconsistency between Chapters 4 and 5 has occurred due to a change in the methodology regarding the evening meal prior to a test day. Specifically, no standardised evening meal was provided to participants in Chapter 4, whereas this meal was provided to participants in Chapter 5. Therefore, it is plausible that the unexpectedly high glycaemic response produced following the FGP breakfast in Chapter 4 is the result of a second meal effect, whereby the GI of the evening meal prior to testing has augmented the glycaemic response shown after breakfast the following morning (Wolever & Jenkins, 1988). This could have occurred if participants in Chapter 4 had a high GI meal the evening prior to testing, although evening meal consumption was not measured so this cannot be confirmed with the present data. Thus, the comparably

lower glycaemic response following the FGP breakfast in Chapter 5 could be due to the implementation of a standardised evening meal prior, which removes the potential of a confounding second meal effect on glycaemic response during testing.

The comparison between good and poor glucoregulatory groups within all samples indicated that poor glucose regulators produced a significantly higher glycaemic response overall compared to the good glucose regulators. For Chapters 4 and 6, a significant Time by Regulator Type interaction revealed that the poor glucose regulators produced a significantly higher glycaemic response for the majority of the day compared to the good glucose regulators. Findings from Chapter 5 indicated that poor glucose regulators produced a significantly higher glucose concentration overall compared to good glucose regulators but the two groups did not significantly differ at any particular assessment across the day. Therefore, it was clear throughout testing that glucose tolerance had a moderating effect on the glycaemic response profile that was produced across the day to the test meals. It is likely that the lack of a significant Time by Regulator Type interaction during Chapter 5 reflects comparatively better glucose tolerance within the younger sample implemented (clinically healthy, 18-25 years) compared to Chapter 4 (clinically healthy, 18-65 years). Previous research has indicated an association between poorer glucose tolerance and older age due to a progressive deterioration of multiple underlying mechanisms in clinically healthy individuals (see Chapter 2). Therefore, it is likely that the Chapter 5 sample did not significantly differ in glycaemic response at any assessment throughout the day due to comparatively better glucose tolerance than the older clinically healthy sample implemented in Chapter 4. However, given the overall significantly higher glucose concentration produced by poor glucose regulators compared to good glucose regulators in Chapter 5, a moderating effect of glucose tolerance on glycaemic response is still clear in a younger clinically healthy sample, albeit to a lesser extent than Chapters 4 and 6.

7.1.2 Cognitive Performance

7.1.2.1 The impact of the glycaemic conditions on cognitive performance

The impact of the glycaemic conditions appears to vary dependent on the sample being investigated in the present thesis. For instance, in Chapter 5 where a clinically healthy sample was examined, it was found that participants were significantly quicker to react on average in the UGP condition compared to the FGP condition during Merged CRT performance. The only other significant finding from Chapter 5 was that good glucose regulators reacted significantly quicker on the LM task immediately after lunch in the UGP condition compared to the FGP condition. These findings may reflect a higher neural glucose availability from the UGP meals during cognitive assessments, which may occur due to a typically quicker glucose release rate of HGI meals (Jenkins et al., 1981). The findings from Chapter 6 indicated that participants were significantly more accurate on the RVIP task immediately before lunch in the FGP condition but were significantly more accurate on the CRT task immediately after lunch in the UGP condition. These findings may also be explained by the different glucose release rates of LGI and HGI meals (Jenkins et al., 1981). For example, a slower release of glucose into the bloodstream from the FGP breakfast could have provided the brain with a steadier supply of

glucose throughout the postprandial period, which may explain why significantly better RVIP accuracy was seen at the last testing point before lunch, where differences in neural glucose availability between glycaemic conditions could be at their largest. Using the same logic, a quicker glucose release rate from the UGP lunch may lead to a comparatively higher neural glucose availability during the first cognitive assessment post lunch, which could explain the significantly higher CRT accuracy in the UGP condition at this point. Interestingly, Chapter 6 also found that FGP breakfast consumption was associated with sustained performance on all three global measures (i.e. accuracy, reaction time and performance) across the morning, whereas the UGP breakfast appeared to be detrimental to performance. This finding is in line with previous research that has frequently reported a LGI breakfast meal to be associated with sustained performance across the morning (see Chapter 2 for a review).

When comparing the cognitive findings across Chapters 5 and 6, it appears that the glycaemic conditions had a greater impact on cognitive performance in those with T2DM (Chapter 6) compared to the clinically healthy (Chapter 5). It can be seen that the clinically healthy sample (Chapter 5) only appears to benefit from the UGP glycaemic intervention under a higher cognitive load (i.e. Merged CRT performance and LM task performance). This is likely due to these tasks having a higher cognitive demand than the simpler tasks, which would require a higher neural glucose uptake (see Chapter 2 for mechanisms). Whereas, the T2DM sample (Chapter 6) appears to benefit from both glycaemic interventions on the simpler tasks (i.e. CRT and RVIP task performance), as well as Global Cognitive measures, particularly across the morning. Considering that both samples followed the exact same testing protocol, it is likely that this inconsistency of cognitive benefits between studies has occurred due to the difference in glucose tolerance status between the samples. Indeed, previous research has indicated that those with poorer glucose tolerance (e.g. T2DM) are more sensitive to glycaemic interventions than the clinically healthy (Lamport et al., 2009). In the present thesis, the comparison of findings between Chapters 5 and 6 highlights that those with poorer glucose tolerance (i.e. T2DM) stand to gain more cognitive benefits from glycaemic interventions during a three meal testing paradigm across the day. This is likely due to more injury occurring in those with poorer glucose tolerance to underlying mechanisms such as BBB glucose transport rates (see Chapter 2 for mechanisms), which would make them more susceptible to glycaemic interventions. Thus, in theory, glycaemic interventions which increase and sustain blood glucose concentrations over a longer period (e.g. FGP condition), and in turn increase BBB glucose transport rates in order to maintain the neural-blood glucose ratio (see Chapter 2), would have a greater effect on cognitive performance in those with poorer glucose tolerance compared to the clinically healthy as the findings across the present studies suggest.

Overall, the glycaemic conditions appear to have had minimal impact on cognitive performance in the clinically healthy throughout the present thesis, although there was some indication that LGI meal consumption can generally benefit cognitive performance in those with T2DM. It is plausible that the matching of energy and macronutrient contents between test meals has reduced the number of significant cognitive differences between glycaemic conditions. For example, Micha et al. (2011) failed to match energy and macronutrient contents between conditions and found improved performance on measures of attention after HGI meal consumption. Indeed, there have been many previous studies that have reported significant cognitive differences between two or more glycaemic conditions, where

energy and macronutrient contents have not been matched (see Chapter 2). It has also been previously indicated that fat and protein share a negative relationship with postprandial glucose rise, which could affect the supply of glucose to the brain from meal consumption (Jenkins et al., 1981). Thus, it is plausible that Micha et al. (2011) identified the HGI condition as more beneficial to cognitive performance due to confounding effects of macronutrient mismatching between conditions. It is therefore logical that the present thesis avoided these confounding effects by accurately matching the energy and macronutrient contents between test meals, which could explain why there were minimal significant cognitive differences between glycaemic conditions.

7.1.2.2 The impact of glucose tolerance on cognitive performance

The findings across this thesis indicate that glucose tolerance has a significant impact on cognitive performance both within and between clinically healthy and T2DM samples. In Chapter 5, it was found that good glucose regulators within the clinically healthy sample were significantly more accurate on the Merged and Merged RVIP measures compared to the poor glucose regulators in the FGP condition. However, it was also observed that the poor glucose regulators reacted significantly quicker than good glucose regulators during Merged RVIP performance. The finding that poor glucose regulators were significantly quicker to react but less accurate during Merged RVIP performance suggests that the poor glucose regulators were simply committing an accuracy-reaction time trade-off where one component is sacrificed for improvements in the other (Bogacz et al., 2010; Fitts, 1966; Reed, 1973; Wickelgren, 1977; Wood & Jennings, 1976). However, the findings suggest that the good glucose regulators benefitted more from the FGP meals than the poor glucose regulators on the Merged task accuracy measure. Given that both glucoregulatory groups consumed the same meals, this finding likely reflects the underlying effects of glucose tolerance on cognitive performance. For example, higher insulin resistance is associated with a slower neuronal glucose uptake rate (see Chapter 2). Therefore, it is plausible that while the FGP meals deliver glucose into the bloodstream at a steady rate for the two glucoregulatory groups, the good glucose regulators may utilise this glucose more efficiently through quicker glucose uptake by neuronal cells, which may explain the higher Merged accuracy observed in this group. Interestingly, Chapter 6 found good glucose regulators within the T2DM sample were significantly more accurate overall during both Merged CRT and RVIP performance compared to the poor glucose regulators. These findings support the proposal that the presence of poorer glucose tolerance within a sample has an adverse effect on cognitive performance, with one potential mechanism being a less efficient glucose utilisation by the neuronal cells (see Chapter 2 for mechanisms). Chapter 6 also found that poor glucose regulators were significantly quicker to react on the CRT task, which suggests there were minimal differences in psychomotor slowing between the glucoregulatory groups within the T2DM sample.

Overall, the cognitive findings indicate that poorer glucose tolerance within a clinically healthy or T2DM sample can have an adverse impact on cognitive performance, although this appears minimal. However, when comparing findings across Chapters 5 and 6, a consistent finding was that task difficulty plays a moderating role in the relationship between glucose tolerance and

cognitive performance. Indeed, it was observed that good glucose regulators outperformed poor glucose regulators under a higher cognitive load (e.g. Merged RVIP performance) but did not significantly differ on simpler tasks (CRT task) across studies. A potential explanation for this is a difference in injury to underlying mechanisms which associate glucose tolerance and cognitive performance, such as neural glucose uptake rates, that become more stressed under a higher cognitive load (see Chapter 2). In other words, the findings suggest that a greater task difficulty is more likely to expose increased injury to underlying mechanisms, such as reduced neural glucose uptake rates, in those with comparatively poorer glucose tolerance in the form of poorer cognitive performance. Furthermore, the fact that this finding was consistent across studies suggests that even minimal differences in injury to underlying mechanisms between glucoregulatory groups in the clinically healthy (Chapter 5) can be exposed through increased task difficulty. Although no statistical comparison between the two samples was conducted in this thesis, previous research has indicated that psychomotor slowing, often displayed by T2DM individuals when compared to clinically healthy controls, may be a manifestation of central neuropathy which is induced by chronic hyperglycaemia, characteristic of T2DM (ADA, 2013; Ryan et al., 1992; Ryan & Geckle, 2000).

7.1.3 Subjective Mood

7.1.3.1 The impact of the glycaemic conditions on subjective mood

The analysis of subjective mood data indicated that no significant differences between conditions were observed in the three studies and post-hoc analysis conducted here for any of the three mood factors; alertness, anxiety and contentment. This finding suggests that an acute manipulation of the glycaemic response through GI variation has minimal impact on subjective mood outcomes across the day. However, it is also possible that ratings of subjective mood may have been affected by perceived social influence. For example, Bowling (2005) highlights that self-reported scores can be exaggerated or minimized if a questionnaire is completed in front of other people due to social beliefs or expectations, leading to different responses compared to administration via post or internet. Whilst participants completed questionnaires alone in the room away from other people, it is still plausible that they felt the need to avoid displaying negative affect to the researcher. Thus, it is possible that participants could have exaggerated their answers on scales they deemed as positive traits (e.g. happy vs. sad) and minimized their answers on scale deemed as negative traits (e.g. mentally slow vs. quick witted). Therefore, it is plausible that the method of subjective mood assessment (i.e. completing mood questionnaires in a strict testing environment, knowing the questionnaire will be handed to the researcher after testing) could have influenced a participant's answers. At present, there is no way to confirm this theory with the present data, although it may be possible for future work to obtain subjective measures of "perceived social influence" of the researcher's nearby presence and the testing environment after a participant has completed all test visits and include these measures as covariates during statistical analyses. Finally, it is also plausible that the glycaemic conditions had significantly different immediate impacts on subjective mood that were missed due to the timing of subjective assessments. In the present studies, subjective assessments took place one hour prior to, as well as fifteen minutes post, meal consumption. If subjective assessments had instead been measured immediately before

and after each meal, then the immediate impact of each glycaemic condition could have been examined and compared. Thus, it is possible that the two conditions could have had a significantly different immediate impact on subjective measures, but this cannot be confirmed by the present data.

7.1.3.2 The impact of glucose tolerance on subjective mood

The comparison between glucoregulatory groups within each sample revealed no significant differences between good and poor glucose regulators on any of the three mood factors in both Chapters 5 and 6. In fact, the only significant difference between glucoregulatory groups was observed during Chapter 4 where poor glucose regulators were significantly more anxious overall compared to good glucose regulators. Given that the sample investigated in Chapter 4 had a larger age range (18-65 years), it is plausible that glucose tolerance varied to a larger degree between participants within the NGT range compared to the younger sample investigated in Chapter 5 (18-25 years). Indeed, the glycaemic screening data indicated that the fasting glucose range of Chapter 4 participants was 3.8-5.7mmol/L whilst it was 4.2-5.7mmol/L in Chapter 5. Although, this difference appears minimal, injury to underlying mechanisms associating glucose tolerance and subjective mood may vary to a greater extent in the Chapter 4 participants (see Chapter 2). For example, the good glucose regulators in Chapter 4 may have suffered no injury to HPA function, whereas the good regulators in Chapter 5 may have suffered some but minimal injury to HPA function. This could result in circulating cortisol levels varying to a greater degree between glucoregulatory groups in Chapter 4 compared to Chapter 5, which could explain why poor glucose regulators are comparatively more anxious compared to good glucose regulators in Chapter 4 only (Reagan et al., 2008). Overall, the mood findings indicate that glucose tolerance had a minimal impact on subjective mood ratings and that negative affect was not associated with the presence of T2DM in the present thesis.

7.1.4 Subjective ratings of Hunger, Fullness and Sleepiness

7.1.4.1 The impact of the glycaemic conditions on subjective hunger, fullness and sleepiness

The analysis of subjective hunger, fullness and sleepiness returned no significant differences between the glycaemic conditions in any of the three studies. This finding likely reflects the accurate matching of energy and macronutrient contents between the test meals. For example, previous research has indicated that acutely increasing the energy content of a meal can increase its' satiating effect and reduce later nutritional consumption (Graff et al., 1992; Kirkmeyer & Mattes, 2000; Lawton et al., 1993). Furthermore, it has been previously reported that a high protein snack had a greater satiating effect than a high fat or high CHO snack and delayed the onset of the subsequent meal (Marmonier, Chapelot & Louis-Sylvestre, 1999). A high protein meal has also been reported to decrease subsequent energy intake during the following meal more than a high fat or CHO meal (Johnson & Vickers, 1993). Interestingly, macronutrient variation between meals has also been reported to affect subjective sleepiness, with a high-fat low-CHO meal associated with higher sleepiness levels during the post prandial

phase compared to a low-fat high-CHO meal (Wells et al., 1997). Furthermore, the consumption of a meal has been previously reported to be associated with a longer duration of sleep during the postprandial phase compared to meal omission (Zammit et al., 1995). Taken together, these previous findings indicate that a meal's impact on subjective hunger, fullness and sleepiness can vary dependent on its energy and macronutrient content. Given that test meals throughout this thesis were matched in these areas, it is likely that confounding effects of mismatching has been avoided, which likely explains why there were consistenly no significant differences between glycaemic condition for subjective hunger, fullness and sleepiness across the present studies.

7.1.4.2 The impact of glucose tolerance on subjective hunger, fullness and sleepiness

Glucose tolerance appears to have had a minimal impact on ratings of subjective hunger, fullness and sleepiness when comparing glucoregulatory groups within each healthy sample. Specifically, Chapter 5 found no significant differences between good and poor glucose regulators on any of these measures whilst Chapter 4 only found that poor glucose regulators were significantly hungrier immediately after lunch and snack consumption in the UGP condition compared to good glucose regulators. Interestingly, hyperglycaemia has been previously reported to be associated with increased hunger levels (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997; Srinivasan & Ramarao, 2007; Triplitt, 2012; Nakamura, 1962). Therefore, it is plausible that the poor glucose regulators are feeling significantly hungrier than the good glucose regulators in Chapter 4 due to longer periods of hyperglycaemia, which is supported by the glycaemic data (see Chapter 4). It is also plausible that the poor glucose regulators secret insulin at a comparatively slower rate than the good glucose regulators. Previous research has indicated an association between elevated insulin levels and reduced production of the hunger stimulating hormone ghrelin (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014; Taheri et al., 2004). This would offer a plausible explanation as to why the significant hunger differences between glucoregulatory groups in Chapter 4 occurred immediately after lunch and snack consumption, where initially different rates of insulin secretion may have a greater effect on ghrelin production compared to circulating insulin levels later in the postprandial phase. Interestingly, Chapter 6 found that poor glucose regulators within the T2DM sample felt significantly fuller and less hungry compared to good glucose regulators during the UGP condition. It is plausible that reduced insulin sensitivity in the poor glucoregulatory group may result in circulating insulin levels remaining elevated for a longer duration compared to the good glucoregulatory group (ADA, 2013). As elevated insulin levels have been associated with reduced secretion of the hunger-stimulating hormone ghrelin (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014), this could result in the lower subjective hunger ratings reported by the poor glucoregulatory group in the UGP condition. This difference may only be occurring in the UGP condition due to the expectedly higher insulin responses that follow a higher glycaemic response, elicited from consumption of a higher GI meal (O'Dea, Nestel & Antonoff, 1980; Holt, Brand-Miller & Petocz, 1997; Jenkins et al., 1981).

Overall, the present findings across this thesis suggest that poorer glucose tolerance, especially T2DM, can affect ratings of subjective hunger, fullness and sleepiness in an acute setting. One potential mechanism that may underpin this effect could be the dysregulation of hormones, such as ghrelin, brought about by increased insulin resistance and reduced insulin secretion. However, when comparing the subjective findings across studies it is also important to consider the potential confoundings effects of age. Indeed, previous research has indicated that biological ageing is associated with a reduction in both appetite and food intake (Visvanathan, 2003). One proposed mechanism for this association is an impairment of gastrointestinal sensory and motor functions with ageing, which results in a more rapid filling and distension of the stomach antrum, for any given gastric volume (Kupfer et al., 1985; MacIntosh, Morley & Chapman, 2000; Rayner et al., 2000). In context of the present studies, poorer glucose tolerance in the older T2DM sample (Chapter 6, 40-70 years) resulted in feeling fuller and less hungry than those with comparatively good glucose tolerance. Whereas, in the younger clinically healthy sample (Chapter 4, 18-65 years) poorer glucose regulators reported higher levels of hunger than comparatively better glucose regulators. Taken together, these findings suggest that that older age and poorer glucose tolerance can have an synergistic effect on subjective measures, such that the effect of one on subjective measures is lessened without the presence of the other.

7.2 Other important findings from this thesis

7.2.1 The impact of task difficulty on cognitive performance

Increases in task difficulty had a clear adverse impact on cognitive performance throughout this thesis. Indeed, the comparisons between the CRT and RVIP tasks with performance on their respective aspects within the Merged tasks consistently displayed performance drops. Specifically, participants were significantly less accurate (Chapter 6) and reacted significantly slower (Chapters 5 & 6) during Merged CRT performance compared to the CRT task. Furthermore, participants were significantly less accurate (Chapters 5 & 6) and reacted significantly slower (Chapter 6) during Merged RVIP performance compared to the RVIP task. Taken together, these findings suggest that the increased cognitive load of the Merged task consistently resulted in poorer cognitive performance across this thesis. As aforementioned, the majority of cognitive findings within each sample indicated that the good glucose regulators outperformed the poor glucose regulators on the more difficult tasks, particularly on accuracy measures during Merged CRT and RVIP performance (see Section 5.1.2.2). Previous research has indicated that neural glucose demand is higher during an increased cognitive load (Chen et al., 1993; McNay et al., 2000). Thus, it is likely that an increased cognitive load such as the Merged task here increases the neural glucose demand of participants whilst performing the task. Therefore, an inability to adequately match this demand over the duration of the Merged task could have resulted in the poorer performance displayed by the comparatively poor glucoregulatory groups. This finding suggests that the adverse cognitive effects of injury to underlying mechanisms associating poorer glucose tolerance and cognitive impairment become more apparent under an increased cognitive load (see Chapter 2 for mechanisms).

In addition, an increased task difficulty seemed to increase the likelihood of observing practise effects whereby participants became more proficient at the task with repeated exposure (Bell et al., 2018). Indeed, it was observed that participants generally became more accurate (Chapter 6) and reacted quicker (Chapter 5) at each subsequent assessment on the LM task throughout the day. Overall, the findings indicate that an increased cognitive load was detrimental to cognitive performance and a more difficult task required more exposures to gain proficiency and understanding across the day. The findings also indicate that those with poorer glucose tolerance are more likely to display cognitive impairments under an increased cognitive load, which could reflect to adequately match increasing neural glucose demand.

7.2.2 Second meal cognitive effects

Previous research has indicated that the GI of one meal can affect cognitive performance following the next subsequent meal (Lamport et al., 2011). Indeed, Lamport et al. (2011) found that a HGI evening meal was associated with better cognitive performance following a standardised breakfast meal the following day. However, no other studies have reported evidence of this effect to date. Interestingly, there was some suggestion of a potential second meal cognitive effect during Chapter 6. Specifically, consistent improvements to LM Reaction Time and Global Cognition Reaction Time was observed after UGP lunch consumption with these improvements continuing post snack. In addition, Chapter 6 also observed consistent improvements to LM Reaction Time after FGP breakfast consumption, which continued post lunch. These findings suggest that the UGP lunch and FGP breakfast are producing a beneficial second meal cognitive effect, which is observed following the relevant subsequent meal. As aforementioned, the current literature has only reported evidence of a second meal practise effect occurring in a dinner-breakfast paradigm, making the current finding of the effect occurring during the day important. However, further testing of cognitive performance and these meals, with the addition of a standardised subsequent meal, would be required to confirm the presence of a second meal cognitive effect. For example, researchers could implement a cross-over design where participants consume both the FGP and UGP breakfast meals, followed by a standardised lunch meal. If better cognitive performance was still observed after lunch in the FGP condition, then the presence of a beneficial second meal cognitive effect from the FGP breakfast could be confirmed.

7.3 Implications of the findings

T2DM is among the most prevalent and chronic diseases in the world. It constitutes the body's inability to produce enough insulin as well as not being able to effectively use the insulin that it does produce. With the number of cases rapidly rising, it was previously predicted that there would be 366 million people worldwide suffering from T2DM by 2030 (Wild et al., 2004). However, a global report on T2DM released by the WHO (2016) indicated that 422 million people suffered from T2DM in 2014, compared to 108 million in 1980. This highlights the ever increasing rate of those developing T2DM, and the rapidly rising global burden of the disease. It is estimated that the global annual cost of diabetes-related treatment is in excess of $827

billion (Seuring, Archangelidi & Suhrcke, 2015). The WHO (2016) also reported that 1.5 million deaths caused by type 1 and 2 diabetes combined occurred in 2012, with higher-than-optimal blood glucose causing an additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases. Whilst the majority of people suffering from T2DM are adults, there has been an increasing rate of children suffering from the disease (Koopman et al., 2005; WHO, 2016). It is already recommended that those with T2DM consume a low GI/GL diet in order to improve glycaemic control (ADA, 2008; Brand-Miller et al., 2003; Dyson et al., 2011; Evert et al., 2014). The novel approach taken in this thesis of implementing a three meal testing paradigm, using representative meals of the daily diet, has highlighted the large differences in glycaemic response that can occur when consuming a LGI or HGI diet across a single day in both clinically healthy and T2DM individuals. Thus, the findings in this thesis imply that the consumption of a LGI diet can indeed improve glycaemic control and that measurable differences to the glycaemic response can be seen immediately in an acute setting. By consuming a LGI diet, it is possible that a healthy individual's risk of developing T2DM would be lower, which could reduce the global burden on health care costs as well as extending the individual's life expectancy. A person suffering from T2DM would also benefit from the consumption of a LGI diet as improved glycaemic control may also lead to a reduction in associated complications such as cardiovascular diseases, which would benefit the individual's health and further reduce global health care costs.

The link between poorer glucose tolerance, such as T2DM, and cognitive impairment is well known (see Chapter 2). Given the decreasing age of T2DM onset, it is possible that cognitive impairment associated with T2DM may become more apparent in younger individuals globally. This raises the important question of whether cognitive impairment in those with poorer glucose tolerance can be attenuated, or even reversed, through glycaemic interventions. Previous research up until this point has investigated this question over the course of a single meal, which breakfast being largely the only meal to be explored. However, the novel approach of exploring three consecutive meals across the day presented in this thesis highlights differential effects of two glycaemic conditions on cognitive performance dependent upon glucose tolerance status. Specifically, the glycaemic conditions appear to have had very little impact in the clinically healthy sample (see Chapter 5). However, the T2DM sample displayed improvements to psychomotor function from HGI meal consumption but better sustained attention performance from LGI meal consumption. There was also some indication that LGI meal consumption benefitted overall cognitive performance in the T2DM sample. These findings support the previous proposal that those with poorer glucose tolerance, such as T2DM, stand to gain more cognitive benefits from glycaemic interventions (Lamport et al., 2009). This is an important finding as it demonstrates a higher sensitivity to diet GI variation in those with T2DM, which can have both glycaemic and cognitive connotations. It was also found that those with T2DM display evidence of psychomotor slowing, which has previously been considered a common manifestation of central neuropathy brought about by chronic hyperglycaemia, characteristic of T2DM (ADA, 2013; Ryan et al., 1992; Ryan & Geckle, 2000). This finding further highlights the importance to maintain good glycaemic control throughout the life span, in an effort to avoid cognitive deficits such as psychomotor slowing. Interestingly, the majority of cognitive findings indicated that the good glucoregulatory group outperformed the poor glucoregulatory group within each sample

during more difficult tasks such as the Merged task. Therefore, the present findings also imply that the adverse cognitive effect of poorer glucose tolerance becomes more apparent under an increased cognitive load. Considering that this thesis explored a fully representative daily diet across three consecutive meals, the real world impact of this finding could be that an individual with poorer glucose tolerance (e.g. T2DM) may display more cognitive impairment when under a heavy wworkload which could adversely affect the individual's efficiency and work output. Finally, the findings from this thesis imply that the presence of T2DM can have a significant impact on subjective mood, potentially through the associated dysregulation of neurotransmitters and hormones. Overall, this thesis demonstrates that LGI meal consumption can acutely benefit glycaemic control in both clinically healthy and T2DM individuals through the novel approach of exploring a three meal testing paradigm across the day. Furthermore, this the findings from thesis support the proposal that those with poorer glucose tolerance are more sensitive to glycaemic interventions than the clinically healthy (Lamport et al., 2009) and indicate that overall cognitive performance in those with T2DM can particularly benefit from LGI meal consumption. In order to further our knowledge of the relationship between glycaemic response, cognitive performance and subjective mood, it is recommended that the novel approach of exploring a three meal testing paradigm, presented here, be either implemented or extended in future research, rather than reverting to the previously exhausted investigation of a single meal.

7.4 Mechanisms of action

There are a number of underlying mechanisms which associate glucose tolerance and cognition (see Chapter 2). The purpose of this thesis was not to investigate these mechanisms, which consequently means that the findings do not provide support for any specific mechanism. However, as discussed in Chapter 2, there are a number of mechanisms which may explain findings reported throughout this thesis, which warrant consideration.

7.4.1.1 Glucose transport across the blood brain barrier (BBB)

Previous research indicates that the rate at which glucose crosses the BBB is reduced in those with abnormal glucose tolerance, such as T2DM (Convit, 2005). The current evidence indicates that this may be the result of BBB endothelium dysfunction (Baron, 1996; Benatti et al., 2016; Brownlee, 2001; Cohen, 1993; Huber et al., 2008; Su et al., 2008) and a subsequent reduction in GLUT 1 transporter availability and function (Duelli et al., 2000; Hwang et al., 2017; McCall, 1992; Mooradian & Morin, 1991; Prasad et al., 2014; Shah, DeSilva & Abbruscato, 2012). Interestingly, neural activity has also been reported to be an important determinant of neuronal glucose uptake in normoglycaemic conditions (Messier, 2004). Indeed, previous reviews of the literature have continuously highlighted the importance of cognitive load as a moderator in the relationship between glycaemic response, glucose tolerance and cognitive performance (Boyle et al., 2018; Sunram-Lea & Owen, 2017). The present findings within this thesis have indicated that poorer glucose regulators perform worse on cognitive tasks which involve a higher cognitive load such as the Merged and LM tasks compared to good glucose

regulators. Therefore, it is possible that these findings may reflect a comparatively reduced rate of neuronal glucose uptake in those with poorer glucose tolerance, which becomes more evident under higher cognitive demand. This would also offer a plausible explanation as to why glucose consumption is often reported as most beneficial to those with poorer glucose tolerance (Awad et al., 2002; Lamport et al., 2009).

7.4.1.2 Neuroinflammation

BBB dysfunction can also lead to increased permeability of the BBB, with a recent research proposing that this increases neuroinflammatory burden (Benatti et al., 2016 De Felice & Ferreira, 2014). The increased BBB permeability then allows plasma components, immune molecules and cells to enter the brain (Abbot et al., 2010; da Fonseca et al., 2014). These components can then activate resident microglia, which are non-neuronal cells involved in the immune defence of the brain (Abbott & Friedman, 2012; Delpech et al., 2015; Skapr et al., 2014). Repeated activation of these microglia can lead to more severe responds to each subsequent disruption of the brain environment (Skaper, Facci & Giusti, 2014), and an over production of pro-inflammatory cytokines (Zunszain et al., 2011). Examinations of the blood from those with T2DM have indicated elevated levels of pro-inflammatory cytokines (Boni-Schnetzler et al., 2008; Chen et al., 2007; Hivert et al., 2009). The result of this process is an increased neuroinflammatory burden that has been shown to adversely affect the structure and function of neuronal cells, which could have cognitive consequences (Benatti et al., 2016). It is plausible that different extents of neuroinflammation, even if minimal, have occurred between glucoregulatory groups within each sample, which may become more apparent under an increased cognitive load.

7.4.1.3 Neurotransmitters

The dysfunction of glucose transport across the BBB can also have negative connotations for the synthesis and regulation of some neurotransmitters. A dysregulation of neurotransmitters can offer a partial explanation for the relationship between glucose tolerance, cognitive performance and mood (Awad et al., 2004). For example, acetylcholine (ACh) synthesis requires acetyl coenzyme A (acetyl-CoA), which is obtained from glucose (Blass & Gibson, 1979; Gibson et al., 1978). It has been previously indicated that ACh deficits are associated with cognitive impairment (Rush, 1988; Rusted & Warburton, 1989). Therefore, it is plausible that a dysfunction in glucose transfer across the BBB may result in reduced ACh availability and subsequent cognitive deficits. In the present thesis, good glucose regulators within Chapters 5 and 6 performed better on the more complex Merged and LM tasks. It is possible that reduced synthesis of neurotransmitters such as ACh has impair cognitive performance in the poor glucose regulators under higher cognitive loads. This may only occur on more difficult tasks as the brain may be demanding more glucose compared to performing a simpler task (McNay et al., 2000), which would make an inability to efficiently synthesis the required amount of ACh more apparent.

The dysfunction of neurotransmitters may also be an underlying mechanism which associates glucose tolerance and negative affect. For example, insulin resistance has been shown to attenuate insulin-induced excitability in dopaminergic neurons, resulting in decreased dopamine signalling and activity (Kleinridders et al., 2015; Konner et al., 2011). Previous post-mortem research comparing suicide victims to healthy individuals reported reduced concentrations of dopamine in the brain regions that mediate mood, such as the amygdala, in the suicide group (Klimeck et al., 2002). In addition, several neuroimaging studies support the hypothesis that major depression is associated with a reduction in dopamine secretion and activity (D'Haenen & Bossuyt, 1994; Ebert et al., 1996; Shah et al., 1997). The subjective mood findings within this thesis were very limited with the only significant difference between glucoregulatory groups within a sample being observed during Chapter 4 where poor glucose regulators were significantly more anxious overall compared to good glucose regulators. However, this finding is more readily attributed to HPA dysfunction which is discussed below (see Section 5.3.1.4). The lack of significant mood differences between glucoregulatory groups, particularly for subjective contentment, suggests that the dysfunction of neurotransmitters such as dopamine is likely to be minimal in the present participants.

7.4.1.4 The HPA axis and glucocorticoids

The HPA axis is considered the common mechanism that mediates an organism's stress response by regulating release of glucocorticoids such as cortisol (Malenka, Nestler & Hyman, 2009). Previous research has indicated that increased insulin resistance, characteristic of T2DM, has been associated HPA axis dysfunction and subsequently elevated glucocorticoid levels (Bruehl et al., 2007; Chiodini et al., 2007; Godoy-Matos et al., 2006; Joseph et al., 2015; Raff & Magill, 2016; Reagan et al., 2008; Roy, Collier & Roy, 1990). It has been demonstrated that acute and chronic rises in glucocorticoids (e.g. cortisol) can impair cognitive performance in areas such as attention, episodic and spatial memory (Aisa et al., 2006; Horner et al., 1990; Sandstrom et al., 2011; Wolf et al., 2003). Although, it should be noted that the underlying mechanisms that associate abnormal glucose tolerance and HPA axis dysfunction are currently unknown (Chan et al., 2005; Reagan et al., 2008). In the present thesis, it was a common finding that poor glucose regulators performed worse on more complex tasks when compared to good glucose regulators within each sample (Chapter 5-6). It is plausible that the poor glucoregulatory groups produced a comparatively higher levels of glucocorticoids such as cortisol due to a greater degree of HPA axis dysnsfunction, which could have impaired cognitive performance. Interestingly, elevated levels of circulating cortisol have been associated with negative mood outcomes and increased risk of affective disorders such as bipolar disorder and depression (Ellenbogen et al., 2004, 2010; Handley et al., 1980; Van Eck et al., 1996). It has also been demonstrated that primates, humans and rodents that have suffered previous life traumas display HPA axis hyperactivity (Heim et al., 2008; Heim & Nemeroff, 2002; Sanchez et al., 2001). Taken together, these findings suggest that HPA axis hyperactivity may be a persistent neurobiological abnormality that predisposes an individual to depression (Pariante & Lightman, 2008). In the context of the present thesis, Chapter 4 found that poor glucose regulators were significantly more anxious overall compared to good glucose regulators. It is possible that comparatively higher insulin resistance in the poor glucoregulatory group has

resulted in elevated levels of cortisol, which presents itself as the higher levels of anxiety reported by this group compared to the good glucoregulatory group in Chapter 4.

7.4.1.5 The role of insulin

As aforementioned, increased insulin resistance is characteristic of T2DM (ADA, 2013). It is known that insulin receptors exist in the brain and that insulin is absorbed by neural areas (Banks et al., 1997). The highest concentration of insulin receptors has been previously reported to occur on the cell walls of the hippocampus (Craft & Watson, 2004; Dore et al., 1997; Marks et al., 1990; Messier, 2004). However, previous research has indicated that increases in insulin resistance within the brain can result in neuronal cells becoming insulin deficient (Strachan, 2003). Ultimately, this results in a reduced neuronal glucose uptake via insulin-facilitated diffusion. Given the link between insulin and neuronal glucose uptake, it is plausible that increased insulin resistance may lead to cognitive deficits (Geroldi et al., 2005 Messier & Tutenberg, 2005; Stranahan et al., 2008). However, there is no current evidence that poorer glucose tolerance, such as T2DM, directly leads to insulin resistance within the brain, although an association between diabetic states and decreased insulin transport has across the BBB has been previously reported (Banks et al., 1997; Baskin et al., 1985; Kaiyala et al., 2000). Given the presence of insulin sensitive glucose transporters on neural areas it is likely that a reduced amount of insulin reaching the brain via impaired BBB transport could reduce neuronal insulin-mediated glucose uptake (Awad et al., 2004; Choeiri et al., 2002; Reagan et al., 2002). Furthermore, it is also known that insulin aids in the storing of glycogen in the brain (Brown et al., 2002; Gailliot et al., 2008), which suggests that increased insulin resistance could result in depleted glycogen stores in the brain, leading to a reduced ability to synthesise glucose during cognitive demand (Brown, 2004; Gruetter et al., 2003). Therefore, differences in insulin resistance between individuals within this thesis may explain some of the cognitive findings that have been reported. For example, the finding that those with poorer glucose tolerance displayed more cognitive impairment when performing a more complex task (Chapter 5-6) suggests that higher insulin resistance has reduced the amount of insulin being transported into the brain. This would result in reduced insulin-mediated glucose uptake of the neuronal cells as well as an inability to synthesise glucose due to depleted glycogen stores. Both of these adverse effects could be more detrimental to performance under an increased cognitive demand where the brain requires more glucose, which is suggested by the present findings.

7.4.1.6 Glucose levels during cognitive assessment

It is plausible that the underlying mechanisms that associate poorer glucose tolerance and cognitive impairment differ from the mechanisms associating acute glucose level changes and cognitive performance. Indeed, previous research has indicated that glucose concentrations during assessment times are associated with cognitive performance (Awad et al., 2002; Donohoe & Benton, 2000; Riby, 2004). It has also been previously demonstrated that a faster rate of decline in peripheral glucose concentrations is associated with improved cognitive

performance in those with T2DM (Perlmuter et al., 2009). In addition, it has been proposed that the relationship between glucose concentrations and cognition follows a bell shape pattern, with acute hyperglycaemia and hypoglycaemia being detrimental to cognitive performance (Riby, 2004). In the present thesis, the findings do not appear to largely support the proposed bell shaped relationship between glucose concentrations and cognitive performance. For example, in Chapter 5 it was reported that the clinically healthy sample did not generally display cognitive benefits from a particular glycaemic condition, even in the presence of measurably different glycaemic response profiles. Furthermore, in Chapter 6 it was found that the T2DM sample performed better on sustained attention measures and all three global measures after the FGP breakfast compared to the UGP breakfast, although the glycaemic response produced by these meals were remarkably similar. However, Chapter 6 also found that the T2DM sample produced significantly better psychomotor function immediately after the UGP lunch compared to the FGP lunch, while glucose concentrations were also significantly higher in the UGP condition at this point. Given that this finding occurred during the initial increase of glucose concentrations following meal consumption and not during a period of hyper- or hypoglycaemia, this finding provides some support for a bell shaped relationship between glucose concentrations and cognitive performance.

7.5 Limitations of this research

7.5.1 The effects of socio-economic status

In the present thesis, specific measures of IQ and educational status were not carried out nor were they part of the inclusion/exclusion criteria for any of the research conducted here. This means that it is likely that participants within each sample varied in both these measures. This is an important limitation to highlight as previous research has indicated that abnormalities in glucose tolerance are more frequent in lower socio-economic groups (Connolly et al., 2000; Evans et al., 2000; Imkampe & Gulliford, 2010). It has been suggested by Brown et al. (2004) that the increased rate of poorer glucose tolerance in lower socio-economic groups is the result of an increased prevalence of risk factors for T2DM such as smoking, obesity, unhealthy eating behaviours and higher alcohol consumption. Therefore, it is possible that individuals with T2DM may demonstrate cognitive impairments compared to clinically healthy individuals as a result of lower socio-economic status, rather than simply due to differences in glucose tolerance status. It is also important to consider that the glucoregulatory groups within each sample examined here may also display significant cognitive differences due to varied socio-economic status, rather than as a result of glucose tolerance differences. For example, the poor glucose regulators in this thesis may also have a lower socio-economic status compared to the good glucose regulators, which may result in comparatively more cognitive deficits. Although, as IQ and educational status were not measured in this thesis, this theory cannot be investigated further.

7.5.2 The cognitive effects of biological ageing

Whilst there were significant differences between glucoregulatory groups on a number of cognitive measures, it is important to also consider the potential underlying effects of biological ageing on cognition. This is particularly relevant for if the clinically healthy (M=21.43, SD=0.32) and T2DM samples (M=56.88, SD=7.82) were compared as they significantly differed in age. Indeed, the association between biological ageing and cognitive decline is well known, and it can begin as early as twenty in clinically healthy individuals (Deary et al., 2009; Salthouse, 2009). Neurobiological variables that can affect the amount of age-related cognitive decline include the accumulation of neurofibrillary tangles (Del Tredici & Braak, 2008) and concentrations of various brain metabolites (Kadota et al., 2001; Bennati et al., 2016). However, age would not have been included as covariate, as it would be statistically impossible to separate the variance explained solely by age from the variance shared between glucose tolerance status and age. This means that any cognitive differences reported between the two samples could partially reflect different levels of age-related cognitive decline rather than effects solely due to the presence of T2DM.

7.5.3 Habitual diet

Previous nutritional consumption has been shown to influence the postprandial glycaemic response following the next subsequent meal (Wolever, 1988, 2006). In addition to this second meal effect, there is evidence for a potential second meal cognitive effect where the GI of one meal can affect cognitive performance after consumption of the following meal, even after an overnight fast (Lamport et al., 2011). To minimise these potential effects from previous meal consumption, the present research provided participants with a standardised evening meal and compliance to this meal were checked via verbal self-report on the morning of testing. No deviations from this meal were mentioned by any participant. However, a standardised evening meal was not provided in Chapter 4, which may have affected the glycaemic response produced after breakfast consumption. For example, if participants consumed a LGI evening meal before the UGP condition, this may have attenuated the glycaemic response shown after the UGP breakfast. It is also possible that participants could have consumed a HGI evening meal before the FGP condition, which could have augmented the glycaemic response produced following FGP breakfast consumption. Indeed, the glycaemic data from Chapter 4 revealed an unexpectedly high glycaemic response following the FGP breakfast in a clinically healthy sample (18-65 years), not typical of a LGI meal (Jenkins et al., 1981). However, an unexpectedly high glycaemic response following the FGP breakfast was also observed in T2DM individuals (40-70 years) in Chapter 6, where a standardised evening meal was provided. Whereas, a typically lower glycaemic response was observed after FGP breakfast consumption in the young clinically healthy sample (18-25 years) examined Chapter 5, where a standardised evening meal was also provided. Therefore, it is likely that individual differences between the samples such as glucose tolerance status and age had a larger influence on the glycaemic response compared to previous nutritional intake the evening prior to testing. It is also plausible that the apple juice contained within the FGP breakfast augmented the glycaemic response, which is explained further in Section 5.4.6.

Another important component of the participants' habitual diet is their caffeine intake. Previous research has indicated that a higher habitual caffeine intake is associated with better performance on tasks of verbal memory, information processing speed and choice reaction time after controlling for sociodemographic, health and lifestyle variables (Hameleers et al., 2000; Jarvis, 1993; Johnson-Kozlow et al., 2002). The intake of caffeine has also been shown to have a mild stimulating effect on the central nervous system (CNS), leading to increased arousal (Fredholm et al., 1999; Nehlig, Daval & Debry, 1992; Smith, 2002). Given the potential confounding effects of caffeine intake on cognitive and subjective measures, participants were not allowed to consume any caffeinated product during testing, including tea and coffee. However, habitual caffeine intake was not recorded during this thesis. This is an important limitation as it is possible that some participants could have begun to experience caffeine withdrawal during testing, which could have affected cognitive and subjective measures. Previous research indicates that the onset of caffeine withdrawal typically occurs between twelve and twenty-four hours after abstinence from doses as low as 100mg per day (Juliano & Griffiths, 2004). Typical symptoms of caffeine withdrawal include headache, fatigue, decreased energy, decreased alertness, depressed mood and drowsiness. Therefore, it is plausible that performance on both cognitive and subjective measures could have been adversely affected by the sudden abstinence from caffeine that participation required.

7.5.4 Individual differences in gut motor activity

In the present thesis, individual differences in gut motor activity were not considered. Previous research has indicated that the results of an OGTT in healthy subjects can vary with the phase of normal upper gut motor activity occurring at the point of glucose ingestion (Thompson et al., 1982). The authors suggest that this difference is the result of different rates of delivery of the glucose solution to the absorptive surface of the small intestine. In the context of this thesis, it is possible that differences in gut motor activity between individuals and glucoregulatory groups could affect cognitive and subjective measures. For example, a higher upper gut motor activity following meal consumption could result in a quicker emptying of the stomach. In turn, a quicker emptying of the stomach could lead to a faster increase in ghrelin production, which may present itself as the increased hunger during any given assessment. If a glucoregulatory group contained more individuals with a higher upper gut motor activity, this could potentially result in this group reported significantly higher levels of subjective hunger compared to the other glucoregulatory group. This is an important example as a significant difference between glucoregulatory groups could be occurring due to differences in gut motor activity, rather than an result of differences in glucose tolerance.

7.5.5 The use of a glucose composite score

A novel glucose composite score was implemented to determine good and poor glucose regulators within each individual sample. The use of a glucose composite score has previously been proposed to have more ecological validity than the use of a single glycaemic parameter, as it encompasses the entire glycaemic response (Lamport et al., 2009). However, there are

limitations to its use. For example, the glucose composite score implemented here was retrospectively calculated using the glycaemic data that was recorded during testing. Therefore, interpretations of the glycaemic data are limited as all significant differences between the glucoregulatory groups would be expected given the calculation method. This limits the degree to which glucose tolerance status within each individual sample can be considered a predictor of the glycaemic responses produced. To address this issue, future research could provide participants with a separate standardised meal before either glycaemic condition is carried out. The glucose composite score could then be calculated from the glycaemic responses produced following this standardised meal and participants could be split into glucoregulatory groups before testing. This enable the researcher to examine the predicting power of the glucose composite score and investigate whether participants classed as poorer glucose regulators do indeed produce higher glycaemic responses to test meals than the better glucose regulators. Finally, it should be noted that the glucose composite score, rather than more traditional measures of glycaemic variability (e.g. glycated haemoglobin: HbA1c), was also used to separate T2DM participants into comparatively good and poor glucoregulatory groups (Chapter 6). This means that no officially recognised measure of T2DM severity such as HbA1c was implemented, which limits the interpretation of any significant differences between glucoregulatory groups within the T2DM sample (Chapter 6). Future research could address this issue by measuring HbA1c and separate T2DM individuals into multiple different categories of T2DM severity. This would allow the comparison of more than two groups (i.e. good vs poor glucose regulators) and extend it to multiple groups (e.g. good vs poor vs severe).

7.5.6 Insulin, hormones and neurotransmitters

Throughout this thesis, many of the significant differences in both cognitive and subjective measures have been interpreted as possible reflections of varying levels of insulin, hormones and neurotransmitters between conditions and glucoregulatory groups. For example, the finding from Chapter 6 where poor glucose regulators were significantly fuller and less hungry than good glucose regulators during the UGP condition could be due to increased insulin resistance in the poor glucoregulatory group leading to longer durations of elevated circulating insulin and the associated reduction in secretion of the hunger-stimulating hormone ghrelin (Broglio et al., 2004; Caixas et al., 1902; Chabot et al., 2014). However, it is important to note that insulin, hormones and neurotransmitters were not measured in any of the studies conducted here and thus any interpretations of findings that involve these measures cannot be confirmed by the present data. These measures were not included during testing in the current thesis due to practical reasons. For example, the measurement of insulin and hormonal levels would have required repeated cannula blood samples to be taken at every glycaemic assessment across the day. Given the high number of glycaemic assessments (21-23), the short time in between assessments (15-30 minutes) and the need for a research nurse to take each cannula blood sample, this was not possible within the research unit where the nurse was also required to monitor other studies. The measurement of neurotransmitters was also not practical as participants would have been required to wear an electroencephalography (EEG) device on their head for the duration of the day or this device

would have been needed to be constantly removed and then reattached for every cognitive and subjective assessment.

7.5.7 The impact of T2DM medication

In the present thesis, T2DM participants were instructed to follow their normal medication routines during both conditions. For clarity, only Study 3 (Chapter 6) investigated those with T2DM. However, the medication taken by the T2DM sample may have affected cognitive performance. Previous research has indicated that anti-diabetic pharmaceuticals can improve cognitive performance (Gradman et al., 1993; Hanyu et al., 2009; Herath et al., 2016; Meneilly et al., 1993; Ryan et al., 2006). Therefore, it is possible that cognitive impairments which may be present in the investigated T2DM individuals are reduced by the use of medication throughout the studies in this thesis. However, the majority of individuals diagnosed with T2DM receive some form of medication, which means that the inclusion of these individuals in the present thesis adds more ecological validity to the observed findings. In addition, it would have been unethical to withhold or pause medication during the present research.

7.5.8 The nature of the test meals

The meal profiles implemented throughout this thesis were designed according to the GI concept and matched for energy and macronutrient content. However, the weight of the meals did vary between glycaemic conditions, which may have affected subjective measures, particularly self-reported hunger and fullness. For example, the FGP snack had a weight of 440g whilst the UGP snack had a much smaller weight of 183g. This large difference in weight suggests that the stomach is emptier after the UGP snack compared to the FGP snack. Previous research has indicated that the hunger stimulating hormone ghrelin is released by the ghrelinergic cells in response to stomach emptying (Dickson et al., 2011; Inui et al., 2004; Meier & Gressner, 2004; Sakata & Sakai, 2010; Schwartz et al., 2000). Therefore, if the stomach is comparatively emptier following the UGP snack it is plausible that more ghrelin is being released compared to FGP snack consumption. If this is the case, then the differences in weight between test meals could have affected subjective ratings of hunger and fullness. A potential solution to this issue could be the addition of water to meals with lower weights to fully match weights between test meals. However, this would then cause an imbalance in hydration status between the two conditions which could influence cognition (see Section 5.4.7).

The glycaemic response produced after each meal followed an expected pattern (based upon the GI concept: Jenkins et al., 1981) in both conditions throughout this thesis. However, the glycaemic response following the FGP breakfast meal was unexpectedly high in both Chapter 4 and Chapter 6. As discussed in these chapters, it is likely that the inclusion of apple juice augmented the glycaemic response following the FGP breakfast. Specifically, the sharp rise in glucose concentrations may have been brought about by rapid absorption of the apple juice due to its liquid form (Burkitt & Trowell, 1977; Jenkins et al., 1981). Whereas, the rapid decline

could occur due to the fructose within the apple juice increasing glucokinase activity, which could lead to increased hepatic glucose uptake and reduced hepatic glucose output (Le & Tappy, 2006; Wolever et al., 2009; Wolf et al., 2002). Thus, future research should consider both the form and content of each individual product when designing mixed meals based upon the GI concept in order to address this issue.

7.5.9 Hydration status

An ethical decision stipulated that participants were freely allowed to drink water at any point during the test days, with no limit on the amount that was allowed to be consumed. However, it is possible that water consumption and subsequent hydration status could have influenced cognitive performance during testing. Previous research has indicated that the consumption of water can improve cognitive function (Edmonds et al., 2013; Lieberman, 2007) whilst dehydration can adversely affect cognitive performance (Edmonds, 2012; Gopinathan, Pichan & Sharma, 1988; Sharma et al., 1986). Interestingly, it has also been reported that glucose enhancement of memory was intensified in thirstier participants (Scholey et al., 2009). In the present thesis, subjective ratings of thirst and quantities of water consumed on an ad libitum water were not recorded. Therefore, it is possible that water consumption during testing could have attenuated any effects of the meal profiles on cognition. However, as participants were able to consume the desired amount of water when thirst arose, the studies have greater ecological validity as it can be assumed that participants were neither too thirsty or over hydrated throughout testing. Conversely, it is plausible that the experimental environment impacted a participants' water consumption. For example, a very thirsty participant may not have been willing to interrupt a cognitive testing battery in order to consume water, even though it was desired. Future research can address this issue by measuring subjective thirst and water consumption amounts. However, it should be noted that employing these measures may influence the amount of water consumed by participants, which has been demonstrated by previous research (Hill et al., 1995; Poppitt et al., 1996; Stubbs et al., 1998, Willet, 2012).

7.5.10 Repeated cognitive testing and order effects

The implementation of a crossover research design is beneficial for its control over individual differences between participants. However, repeated exposure to the cognitive tasks throughout testing will inevitably lead to participants becoming more familiar with the tasks with each subsequent assessment (Bell et al., 2018). Therefore, it is possible that this repeated exposure to the cognitive task battery affected cognitive performance. To reduce the likelihood of potential practise effects, participants were given a familiarisation attempt of the task battery during screening and the order of the glycaemic conditions and cognitive tasks within the battery was counterbalanced. Despite this, there was some evidence of practise effects on the Merged and LM tasks in both Chapter 5 and 6, particularly observed on the reaction time measures where general improvements were observed throughout the day. It is likely that the increased task difficulty of these tasks, when compared to the simpler CRT and RVIP tasks, resulted in participants requiring more exposures to gain proficiency and

understanding. In other words, it is possible that increased task difficulty of the Merged and LM tasks was initially detrimental to performance in the morning but with each exposure participants became more proficient at carrying out the tasks, which presents itself as improving performance throughout the day. Future work could address this issue by providing more familiarisation attempts of the cognitive task battery. However, this may then reduce any effects observed on the simpler tasks if participants reach a very high level of proficiency before testing commences.

7.5.11 Demand characteristics

Throughout this thesis participants were not explicitly informed which condition they were carrying out during a particular test day. However, participants were aware that the study they participated in required them to consume meals which had been designed according to the GI concept. Thus, a major limited of the present research was the transparency of the study manipulation, which could have subsequently affected cognitive and subjective measures. For example, participants may have an expectation as to how healthy a particular food appears and have preconceptions of how they will feel after its consumption. In the context of subjective mood, it is possible that a participant may view fruit (e.g. the apple in the FGP snack) as a healthier food than a sugary product (e.g. the lemon curd yoghurt in the UGP snack), which could lead to higher ratings of subjective contentment brought about by the positive feeling of eating a healthier food. Indeed, a participant may also be more motivated to compensate for the consumption of a food they view as unhealthy and subsequently increase effort during the cognitive task battery. Additionally, it is possible that being in an experimental environment which interrupts daily routine and produces an awareness to being tested may have caused increased effort during the cognitive task battery (Hammersley et al., 2007). Given that subjective ratings of cognitive effort were not measured throughout testing, these theories cannot be confirmed by the present data.

7.6 Future work

Taken together, the studies in this thesis suggest that glucose tolerance plays a moderating role in the relationship between glycaemic response and cognitive performance. The underlying effects of poorer glucose tolerance appear to become more apparent under an increased cognitive load. The findings also suggest that those with poorer glucose tolerance, such as T2DM, stand to gain more cognitive benefits from glycaemic interventions such as LGI meal consumption, which supports previous research (Lamport et al., 2009). However, the cross-sectional nature of the present studies means that they do not provide direct evidence that the progression of deteriorating glucose tolerance leads to increasing cognitive impairment. Whilst a post hoc analysis could have been carried out to compare study samples, the potential confounding effects of age must be considered. For clarity, Chapter 5 had a mean age of 21 years whilst Chapter 6 had a mean age of 57 years. Indeed, the association between biological ageing and cognitive impairment is well known, which means that findings such as psychomotor slowing in a T2DM sample compared to a clinically healthy sample (Ryan &

Geckle, 2000) could partially reflect age-related cognitive decline, rather than solely the underlying effects of T2DM, especially if age is not included as a statistical covariate. Finally, many findings throughout this thesis have been interpreted as reflections of possible differences in areas such as circulating insulin, hormonal and neurotransmitter levels between glucoregulatory groups but these measures were not recorded during testing.

To address these issues, future acute and longitudinal work should directly compare cognitive and subjective measures between glucoregulatory groups that have been matched on parameters such as age, IQ and socio-economic status. In line with the studies conducted within this thesis, future work should look to design either short or long term glycaemic interventions based upon a concept such as GI. The potential glycaemic effects of meal components such as apple juice and its' fructose content should also be avoided so interpretations of any glycaemic and cognitive data can be made with confidence that confounding effects of particular food components, such as fructose, are not present. There should be a focus on extending the current two group testing paradigm within this thesis (i.e. good vs. poor regulators or NGT vs. T2DM) to a multiple group testing paradigm (e.g. NGT vs. IFG vs. IGT vs. T2DM). It would also be possible to further split these groups into subgroups such as comparatively good and poor glucose regulators within each glucose tolerance category. Such investigations would be largely informative as to how each group, and possibly subgroup, responds to glycaemic interventions based upon the concept of GI. Furthermore, such research would also be informative as to the extent at which poorer glucose tolerance is associated with cognitive decline. Longitudinal investigations of this type could also record any improvements or declines in glycaemic control within each group as they participate in a glycaemic condition through measures such as HbA1c. If cognitive performance is also assessed multiple times during such longitudinal investigations it would provide an insight as to whether dietary interventions that improve glycaemic control can also attenuate, or even reverse, any cognitive impairments associated with poorer glucose tolerance. Such research is crucial given the reported earlier onset of diseases such as T2DM in the global population (Koopman et al., 2005, WHO, 2016). Furthermore, the investigation of younger populations means that the association between glucose tolerance and cognitive performance could be examined without the presence of other risk factors for cognitive impairment such as age-related cognitive decline and cardiovascular complications. This would allow a clearer identification of the extent to which specific underlying mechanisms associated with glucose tolerance impact cognition and aid the development of further dietary interventions.

7.7 Overall Conclusions

This thesis examined the relationship between the glycaemic response, cognitive performance and subjective mood, under conditions where LGI and HGI meals were consumed across the day. The work in this thesis identified that the consumption of a LGI diet can have beneficial effects on glycaemic control in both clinically healthy and T2DM samples within a single day. It has also been demonstrated that those with T2DM show greater sensitivity to glycaemic interventions in an acute setting compared to the clinically healthy. The clearest example of this was that the T2DM sample here showed sustained performance on all three global

cognitive measures across the morning following a LGI breakfast, whilst the consumption of a HGI breakfast was detrimental to cognitive performance during the same time frame. Taken together, these findings suggest that both clinically healthy and T2DM individuals can improve glycaemic control within a single day through LGI meal consumption and that the cognitive impairment associated with poorer glucose tolerance can be acutely attenuated from the same dietary intervention. Furthermore, the underlying effects of poorer glucose tolerance on cognitive performance within and between clinically healthy and T2DM samples appeared to become more apparent under an increased cognitive load. This finding suggests that underlying mechanisms associating glucose tolerance and cognitive performance become more stressed as neural demand increases. This further highlights the need for dietary interventions which can improve glycaemic control and potentially attenuate or reverse the cognitive impairment associated with poorer glucose tolerance. This thesis also indicates that glycaemic interventions have minimal impact on subjective mood in an acute setting in both clinically healthy and T2DM samples, although poorer glucose tolerance appears to affect subjective mood, potentially through mechanisms such as HPA dysfunction (see Chapter 2 for mechanisms). The implication of these findings is that LGI meal consumption may aid maintenance of a good state of health, which may reduce the risk of developing T2DM and the associated cognitive and subjective mood impairments. Finally, these findings also imply that the cognitive impairment present in those with T2DM may be attenuated in an acute setting through dietary interventions such as the consumption of LGI meals.

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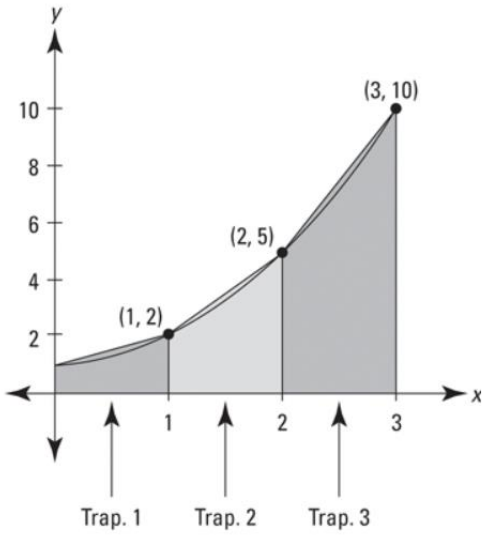
Appendices

Appendix A – The trapezoidal method for AUC calculation

The trapezoidal rule is used to approximate the area under the curve with the following steps.

1 – Trapezoids are drawn on the glycaemic response curve.



* For incremental AUC (iAUC) the x axis is placed where the baseline glucose value is recorded. For example, if a participant's baseline glucose reading was 4.1mmol/L, then the x axis is placed at 4.1 and any area below this is not included in the iAUC calculation. Whereas, the x axis is always placed at 0 when calculating the total AUC (tAUC), meaning all area under the curve is included during calculation, even below baseline readings.

2 – The area of each trapezoid is calculated individually using the following formula.

$$Area = \frac{Sum\ of\ parallel\ sides + height\ of\ trapezoid}{2}\ \text{x}\ change\ in\ x$$

For example, Trap.1's area would be:

$$Area = \frac{1+2}{2}\ \text{x}\ 1 = 1.5$$

Whereas, Trap.3's area would be:

$$Area = \frac{5+10}{2}\ \text{x}\ 1 = 7.5$$

3 – The areas of each trapezoid are added together to give the sum area under the curve.

Appendix B – The cognitive task orders for Studies 2 and 3 (Chapter 5-6)

Table B1: The cognitive task order at every assessment for each participant in Study 2 (Chapter 5).

Subject	1st Condition									2nd Condition								
ID	Base	1	2	3	4	5	6	7	8	Base	1	2	3	4	5	6	7	8
1	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
2	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
3	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
4	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
5	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
6	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
7	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
8	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
9	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
10	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
11	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
12	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
13	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
14	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
15	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
16	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
17	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
18	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
19	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
20	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
21	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
22	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
23	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
24	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
25	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
26	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
27	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
28	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
29	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
30	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
31	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
32	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
33	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
34	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
35	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
36	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A
37	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
38	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
39	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
40	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	A

*A = CRT > RVIP > Merged > LM, B = RVIP > LM > CRT > Merged, C = Merged > CRT > LM > RVIP, D = LM > Merged > RVIP > CRT.

Table B2: The cognitive task order at every assessment for each participant in Study 3 (Chapter 6).

Subject	1st Condition									2nd Condition								
ID	Base	1	2	3	4	5	6	7	8	Base	1	2	3	4	5	6	7	8
1	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
2	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
3	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
4	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
5	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
6	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
7	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
8	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
9	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
10	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
11	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
12	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
13	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
14	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
15	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
16	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
17	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
18	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
19	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
20	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
21	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D
22	B	B	C	D	A	B	C	D	A	B	B	C	D	A	B	C	D	A
23	C	C	D	A	B	C	D	A	B	C	C	D	A	B	C	D	A	B
24	D	D	A	B	C	D	A	B	C	D	D	A	B	C	D	A	B	C
25	A	A	B	C	D	A	B	C	D	A	A	B	C	D	A	B	C	D

*A = CRT > RVIP > Merged > LM, B = RVIP > LM > CRT > Merged, C = Merged > CRT > LM > RVIP, D = LM > Merged > RVIP > CRT.

Appendix C – The Bond-Lader subjective mood questionnaire

Participant ID: Date:

GI & Cognition Study

Mood Questionnaire

1. Please rate the way you feel ***right now*** in terms of the dimensions given below.
2. Regard the line as representing the full range of each dimension.
3. Rate your feelings as they are at this moment.
4. Mark clearly and perpendicularly across each line.

Alert **I----------I** Drowsy

Calm **I----------I** Excited

Strong **I----------I** Weak

Fuzzy **I----------I** Clear-headed

Well-coordinated **I----------I** Clumsy

Lethargic **I----------I** Energetic

Contented **I----------I** Discontented

Troubled **I----------I** Tranquil

Mentally slow **I----------I** Quick-witted

Tense **I----------I** Relaxed

Attentive **I----------I** Dreamy

Incompetent **I----------I** Proficient

Happy **I----------I** Sad

Antagonistic **I----------I** Amicable

Interested **I----------I** Bored

Withdrawn **I----------I** Gregarious

Appendix D – The subjective hunger, fullness and sleepiness questionnaire

Participant ID: Date:

GI & Cognition Study

Appetite and Sleepiness Questionnaire

1. Please rate the way you feel ***right now*** in terms of the dimensions given below.
2. Regard the line as representing the full range of each dimension.
3. Rate your feelings as they are at this moment.
4. Mark clearly and perpendicularly across each line.

Not at all Hungry **I----------I** Very Hungry

Not at all Full **I----------I** Very Full

Not at all Sleepy **I----------I** Very Sleepy

Appendix E – The glycaemic condition orders for Studies 1-3 (Chapter 4-6)

Table E1: The glycaemic condition order for each participant in Study 1 (Chapter 4).

Subject ID	1st Condition	2nd Condition
1	UGP	FGP
2	FGP	UGP
3	UGP	FGP
4	UGP	FGP
5	UGP	FGP
6	UGP	FGP
7	FGP	UGP
8	UGP	FGP
9	FGP	UGP
10	FGP	UGP
11	UGP	FGP
12	FGP	UGP
13	UGP	FGP
14	FGP	UGP
15	FGP	UGP
16	FGP	UGP
17	FGP	UGP
18	FGP	UGP
19	FGP	UGP
20	UGP	FGP
21	FGP	UGP
22	UGP	FGP
23	UGP	FGP
24	UGP	FGP

Table E2: The glycaemic condition order for each participant in Study 2 (Chapter 5).

Subject ID	1st Condition	2nd Condition
1	FGP	UGP
2	UGP	FGP
3	FGP	UGP
4	UGP	FGP
5	UGP	FGP
6	FGP	UGP
7	FGP	UGP
8	FGP	UGP
9	UGP	FGP
10	FGP	UGP
11	FGP	UGP
12	UGP	FGP
13	UGP	FGP
14	FGP	UGP
15	UGP	FGP
16	UGP	FGP
17	FGP	UGP
18	UGP	FGP
19	UGP	FGP
20	UGP	FGP
21	FGP	UGP
22	UGP	FGP
23	UGP	FGP
24	UGP	FGP
25	FGP	UGP
26	FGP	UGP
27	FGP	UGP
28	UGP	FGP
29	FGP	UGP
30	FGP	UGP
31	UGP	FGP
32	FGP	UGP
33	FGP	UGP
34	UGP	FGP
35	UGP	FGP
36	FGP	UGP
37	FGP	UGP
38	UGP	FGP
39	UGP	FGP
40	FGP	UGP

Table E3: The glycaemic condition order for each participant in Study 3 (Chapter 6).

Subject ID	1st Condition	2nd Condition
1	UGP	FGP
2	FGP	UGP
3	UGP	FGP
4	UGP	FGP
5	UGP	FGP
6	FGP	UGP
7	FGP	UGP
8	FGP	UGP
9	UGP	FGP
10	FGP	UGP
11	FGP	UGP
12	UGP	FGP
13	FGP	UGP
14	FGP	UGP
15	UGP	FGP
16	UGP	FGP
17	FGP	UGP
18	UGP	FGP
19	FGP	UGP
20	UGP	FGP
21	FGP	UGP
22	UGP	FGP
23	UGP	FGP
24	FGP	UGP
25	UGP	FGP

Appendix F – LMM results tables

Table F1: LMM results for Study 1 (Chapter 4).

Study 1 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Glucose:					
Glycaemic response	1931.42	Condition (C)	1, 195.43	18.214	< 0.001
		Time (T)	19, 169.44	29.193	< 0.001
		Regulator Type (RGT)	1, 198.67	88.389	< 0.001
		C x T	19, 169.44	6.078	< 0.001
		C x RGT	1, 195.43	3.230	0.074
		T x RGT	19, 169.44	2.606	0.001
		C x T x RGT	19, 169.44	1.361	0.152
		Gender	1, 207.85	5.893	0.016
		Age	1, 207.85	0.487	0.486
		BMI	1, 207.85	1.336	0.249
		Baseline Glucose	1, 207.85	24.471	< 0.001
Mood:					
Alertness	1922.77	Condition (C)	1, 46.19	0.037	0.848
		Time (T)	4, 48	9.196	< 0.001
		Regulator Type (RGT)	1, 48.53	0.004	0.952
		C x T	4, 48	0.418	0.795
		C x RGT	1, 46.57	0.309	0.581
		T x RGT	4, 48	1.015	0.409
		C x T x RGT	4, 48	0.905	0.469
		Gender	1, 48	0.224	0.638
		Age	1, 48	0.168	0.684
		BMI	1, 48	0.083	0.774
		Baseline Glucose	1, 48	0.105	0.748
		Baseline Alertness	1, 48	21.485	< 0.001
Anxiety	1829.5	Condition (C)	1, 46.26	0.597	0.444
		Time (T)	4, 48	2.453	0.058
		Regulator Type (RGT)	1, 47.48	7.013	0.011
		C x T	4, 48	0.988	0.423
		C x RGT	1, 47.02	0.068	0.795
		T x RGT	4, 48	0.506	0.732
		C x T x RGT	4, 48	0.648	0.631
		Gender	1, 48	4.752	0.034
		Age	1, 48	0.041	0.840
		BMI	1, 48	0.038	0.846
		Baseline Glucose	1, 48	0.460	0.501
		Baseline Anxiety	1, 48	61.128	< 0.001

Table F1: Continued.

Study 1 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Mood:					
Contentment	1612.3	Condition (C)	1, 44.72	1.314	0.258
		Time (T)	4, 48	1.968	0.115
		Regulator Type (RGT)	1, 46.70	0.008	0.929
		C x T	4, 48	0.501	0.735
		C x RGT	1, 45.93	4.047	0.050
		T x RGT	4, 48	0.231	0.919
		C x T x RGT	4, 48	0.525	0.718
		Gender	1, 48	1.055	0.310
		Age	1, 48	1.515	0.224
		BMI	1, 48	0.283	0.597
		Baseline Glucose	1, 48	3.042	0.088
		Baseline Contentment	1, 48	152.639	< 0.001
HFS data:					
Hunger	2022.37	Condition (C)	1, 48.27	0.054	0.817
		Time (T)	4, 48	20.413	< 0.001
		Regulator Type (RGT)	1, 50.29	4.570	0.037
		C x T	4, 48	0.243	0.912
		C x RGT	1, 48.06	0.971	0.329
		T x RGT	4, 48	3.162	0.022
		C x T x RGT	4, 48	2.526	0.053
		Gender	1, 48	8.252	0.006
		Age	1, 48	2.469	0.123
		BMI	1, 48	2.046	0.159
		Baseline Glucose	1, 48	0.007	0.935
		Baseline Hunger	1, 48	9.493	0.003
Fullness	2026.82	Condition (C)	1, 41.62	0.002	0.967
		Time (T)	4, 48	33.173	< 0.001
		Regulator Type (RGT)	1, 43.86	3.127	0.084
		C x T	4, 48	0.616	0.653
		C x RGT	1, 41.58	1.459	0.234
		T x RGT	4, 48	2.006	0.109
		C x T x RGT	4, 48	1.066	0.384
		Gender	1, 41.54	4.889	0.033
		Age	1, 46.69	0.009	0.925
		BMI	1, 44.69	2.090	0.155
		Baseline Glucose	1, 47.98	0.815	0.371
		Baseline Fullness	1, 45.04	9.425	0.004

Table F1: Continued.

Study 1 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
HFS data					
Sleepiness	2076.02	Condition (C)	1, 47.09	0.369	0.547
		Time (T)	4, 48	10.400	< 0.001
		Regulator Type (RGT)	1, 48.67	1.629	0.208
		C x T	4, 48	0.373	0.827
		C x RGT	1, 47.15	1.716	0.197
		T x RGT	4, 48	3.768	0.010
		C x T x RGT	4, 48	0.639	0.637
		Gender	1, 48	1.794	0.187
		Age	1, 48	3.164	0.082
		BMI	1, 48	0.478	0.493
		Baseline Glucose	1, 48	0.117	0.734
		Baseline Sleepiness	1, 48	16.264	< 0.001

Table F2: LMM results for Study 2 (Chapter 5).

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Glucose:					
Glycaemic Response	2663.52	Condition (C)	1, 142.23	29.338	< 0.001
		Time (T)	21, 478.25	34.053	< 0.001
		Regulator Type (RGT)	1, 197.37	46.588	< 0.001
		C x T	21, 478.25	7.099	< 0.001
		C x RGT	1, 139.94	0.063	0.803
		T x RGT	21, 478.25	1.230	0.220
		C x T x RGT	21, 478.25	0.750	0.780
		Gender	1, 284.16	3.253	0.072
		Age	1, 284.39	2.343	0.127
		BMI	1, 284.20	0.927	0.336
		Baseline Glucose	1, 284.19	72.122	< 0.001
Cognition:					
CRT Accuracy	2009.38	Condition (C)	1, 80.41	0.027	0.871
		Time (T)	7, 80	0.794	0.594
		Regulator Type (RGT)	1, 96.27	0.418	0.519
		C x T	7, 80	1.018	0.425
		C x RGT	1, 79.95	0.081	0.777
		T x RGT	7, 80	1.089	0.378
		C x T x RGT	7, 80	0.206	0.983
		Gender	1, 80	0.908	0.343
		Age	1, 80	0.695	0.407
		BMI	1, 80	7.158	0.009
		Baseline Glucose	1, 80	0.494	0.484
		Baseline Accuracy	1, 80	44.689	< 0.001
CRT Reaction Time	5783.14	Condition (C)	1, 76.54	0.011	0.919
		Time (T)	7, 80	2.225	0.041
		Regulator Type (RGT)	1, 89.60	1.237	0.269
		C x T	7, 80	2.633	0.017
		C x RGT	1, 75.43	0.044	0.834
		T x RGT	7, 80	1.688	0.124
		C x T x RGT	7, 80	0.697	0.675
		Gender	1, 80	2.395	0.126
		Age	1, 80	2.433	0.123
		BMI	1, 80	0.341	0.561
		Baseline Glucose	1, 80	0.006	0.938
		Baseline Reaction Time	1, 80	136.390	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Cognition:					
RVIP Accuracy	2588.09	Condition (C)	1, 76.65	1.921	0.170
		Time (T)	7, 79	1.805	0.098
		Regulator Type (RGT)	1, 85.33	0.772	0.382
		C x T	7, 79	0.971	0.458
		C x RGT	1, 75.32	0.093	0.762
		T x RGT	7, 79	0.875	0.530
		C x T x RGT	7, 79	1.191	0.318
		Gender	1, 79	0.052	0.821
		Age	1, 79	5.504	0.021
		BMI	1, 79	0.414	0.522
		Baseline Glucose	1, 79	0.082	0.776
		Baseline Accuracy	1, 79	80.619	< 0.001
RVIP Reaction Time	6382.01	Condition (C)	1, 79	2.870	0.094
		Time (T)	7, 79	3.079	0.006
		Regulator Type (RGT)	1, 82.10	0.004	0.949
		C x T	7, 79	0.584	0.767
		C x RGT	1, 78.66	1.640	0.204
		T x RGT	7, 79	1.312	0.256
		C x T x RGT	7, 79	1.159	0.336
		Gender	1, 79	0.191	0.663
		Age	1, 79	2.649	0.108
		BMI	1, 79	0.243	0.623
		Baseline Glucose	1, 79	1.677	0.199
		Baseline Reaction Time	1, 79	148.075	< 0.001
Merged Accuracy	2984.23	Condition (C)	1, 78.99	0.132	0.717
		Time (T)	7, 78.73	1.402	0.216
		Regulator Type (RGT)	1, 84.25	2.629	0.109
		C x T	7, 78.73	0.833	0.563
		C x RGT	1, 78.85	4.360	0.040
		T x RGT	7, 78.73	1.011	0.430
		C x T x RGT	7, 78.73	0.210	0.982
		Gender	1, 79.30	0.793	0.376
		Age	1, 79.05	1.031	0.313
		BMI	1, 78.93	0.819	0.368
		Baseline Glucose	1, 79.41	0.093	0.762
		Baseline Accuracy	1, 79.19	160.942	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Cognition:					
Merged Reaction Time	6235.01	Condition (C)	1, 72.23	1.533	0.220
		Time (T)	7, 72.52	1.930	0.077
		Regulator Type (RGT)	1, 83.02	0.853	0.358
		C x T	7, 72.52	1.357	0.237
		C x RGT	1, 70.95	1.960	0.166
		T x RGT	7, 72.52	0.868	0.536
		C x T x RGT	7, 72.52	0.978	0.454
		Gender	1, 71.72	0.006	0.941
		Age	1, 73.56	0.095	0.759
		BMI	1, 72.12	1.176	0.282
		Baseline Glucose	1, 71.79	0.053	0.819
		Baseline Reaction Time	1, 71.65	146.393	< 0.001
LM Accuracy	2746.88	Condition (C)	1, 80.58	3.662	0.059
		Time (T)	7, 80	1.658	0.131
		Regulator Type (RGT)	1, 85.28	1.193	0.278
		C x T	7, 80	0.602	0.753
		C x RGT	1, 79.41	0.491	0.485
		T x RGT	7, 80	0.664	0.702
		C x T x RGT	7, 80	1.017	0.426
		Gender	1, 80	0.473	0.493
		Age	1, 80	0.201	0.655
		BMI	1, 80	1.811	0.182
		Baseline Glucose	1, 80	0.128	0.721
		Baseline Accuracy	1, 80	140.131	< 0.001
LM Reaction Time	9589.17	Condition (C)	1, 75.79	0.316	0.576
		Time (T)	7, 80	2.189	0.044
		Regulator Type (RGT)	1, 96.02	0.116	0.734
		C x T	7, 80	0.821	0.573
		C x RGT	1, 74.48	1.521	0.221
		T x RGT	7, 80	1.608	0.145
		C x T x RGT	7, 80	2.991	0.008
		Gender	1, 80	0.539	0.465
		Age	1, 80	3.490	0.065
		BMI	1, 80	0.525	0.471
		Baseline Glucose	1, 80	0.364	0.548
		Baseline Reaction Time	1, 80	267.329	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Global Cognition:					
Global Accuracy	916.06	Condition (C)	1, 78.36	2.387	0.126
		Time (T)	7, 80	1.680	0.126
		Regulator Type (RGT)	1, 89.83	0.011	0.915
		C x T	7, 80	0.686	0.683
		C x RGT	1, 77.45	1.956	0.166
		T x RGT	7, 80	1.338	0.243
		C x T x RGT	7, 80	0.345	0.931
		Gender	1, 80	2.084	0.153
		Age	1, 80	0.055	0.815
		BMI	1, 80	2.153	0.146
		Baseline Glucose	1, 80	1.141	0.289
		Baseline Accuracy	1, 80	139.426	< 0.001
Global Reaction Time	808.27	Condition (C)	1, 78.54	1.210	0.275
		Time (T)	7, 80	2.349	0.031
		Regulator Type (RGT)	1, 87.10	0.177	0.675
		C x T	7, 80	0.944	0.478
		C x RGT	1, 77.65	0.650	0.422
		T x RGT	7, 80	1.552	0.162
		C x T x RGT	7, 80	0.526	0.813
		Gender	1, 80	0.011	0.919
		Age	1, 80	6.738	0.011
		BMI	1, 80	0.387	0.536
		Baseline Glucose	1, 80	1.495	0.225
		Baseline Reaction Time	1, 80	253.401	< 0.001
Global Performance	721.65	Condition (C)	1, 79.26	0.199	0.657
		Time (T)	7, 80	1.624	0.140
		Regulator Type (RGT)	1, 88.20	0.143	0.707
		C x T	7, 80	0.842	0.556
		C x RGT	1, 78.30	2.054	0.156
		T x RGT	7, 80	1.642	0.136
		C x T x RGT	7, 80	0.666	0.700
		Gender	1, 80	0.108	0.744
		Age	1, 80	2.860	0.095
		BMI	1, 80	2.530	0.116
		Baseline Glucose	1, 80	1.521	0.221
		Baseline Performance	1, 80	259.041	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Task Comparisons:					
CRT vs. Merged (Percent Correct)	6772.70	Condition (C)	1, 377.81	0.069	0.792
		Time (T)	7, 201.43	0.386	0.910
		Task (Ta)	1, 600.43	111.011	< 0.001
		Regulator Type (RGT)	1, 48106	0.126	0.723
		C x T	7, 201.43	0.233	0.977
		C x Ta	1, 554	0.379	0.538
		C x RGT	1, 392.13	1.132	0.288
		T x Ta	7, 192.64	0.211	0.983
		T x RGT	7, 201.43	0.825	0.567
		Ta x RGT	1, 555.27	0.063	0.802
		C x T x Ta	7, 192.63	0.306	0.950
		C x T x RGT	7, 201.43	0.206	0.984
		C x Ta x RGT	1, 563.64	0.006	0.937
		T x Ta x RGT	7, 192.64	1.193	0.309
		C x T x Ta x RGT	7, 192.63	0.317	0.946
		Gender	1, 446.73	0.941	0.333
		Age	1, 430.50	0.030	0.863
		BMI	1, 445.40	7.988	0.005
		Baseline Glucose	1, 446.32	0.823	0.365
		Baseline Percent	1, 816.35	34.010	< 0.001
CRT vs. Merged (Reaction Time)	11,961.36	Condition (C)	1, 160.03	1.951	0.164
		Time (T)	7, 398.59	0.534	0.809
		Task (Ta)	1, 1071.13	230.334	< 0.001
		Regulator Type (RGT)	1, 180.80	0.113	0.737
		C x T	7, 398.59	0.519	0.820
		C x Ta	1, 673.25	23.378	< 0.001
		C x RGT	1, 158.74	1.897	0.170
		T x Ta	7, 284.31	1.445	0.187
		T x RGT	7, 398.59	0.767	0.615
		Ta x RGT	1, 675.45	1.036	0.309
		C x T x Ta	7, 284.30	0.561	0.787
		C x T x RGT	7, 398.59	0.230	0.978
		C x Ta x RGT	1, 676.93	4.893	0.027
		T x Ta x RGT	7, 284.31	0.742	0.637
		C x T x Ta x RGT	7, 284.30	0.375	0.917
		Gender	1, 201.89	5.769	0.017
		Age	1, 198.04	0.027	0.871
		BMI	1, 198.88	4.440	0.036
		Baseline Glucose	1, 198.95	0.151	0.698
		Baseline Reaction Time	1, 1089.22	554.587	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Task Comparisons:					
RVIP vs. Merged (Percent Correct)	10,033.34	Condition (C)	1, 155.68	1.013	0.316
		Time (T)	7, 343.82	0.727	0.649
		Task (Ta)	1, 839.16	115.502	< 0.001
		Regulator Type (RGT)	1, 190.51	0.895	0.345
		C x T	7, 343.82	0.452	0.868
		C x Ta	1, 645.19	5.880	0.016
		C x RGT	1, 154.12	3.011	0.085
		T x Ta	7, 343.95	0.893	0.512
		T x RGT	7, 343.82	0.194	0.987
		Ta x RGT	1, 636.75	0.158	0.691
		C x T x Ta	7, 343.95	0.189	0.988
		C x T x RGT	7, 343.82	0.269	0.966
		C x Ta x RGT	1, 639.76	6.901	0.009
		T x Ta x RGT	7, 343.95	0.343	0.934
		C x T x Ta x RGT	7, 343.95	0.630	0.731
		Gender	1, 223.21	1.512	0.220
		Age	1, 214.93	15.647	0.000
		BMI	1, 223.37	3.008	0.084
		Baseline Glucose	1, 225.17	0.284	0.595
		Baseline Percent	1, 723.56	459.371	< 0.001
RVIP vs. Merged (Reaction Time)	13,479.42	Condition (C)	1, 331.67	9.151	0.003
		Time (T)	7, 325.54	1.874	0.073
		Task (Ta)	1, 1008.50	21.120	< 0.001
		Regulator Type (RGT)	1, 354.66	8.996	0.003
		C x T	7, 325.54	0.409	0.896
		C x Ta	1, 534.95	0.481	0.488
		C x RGT	1, 335.30	13.985	< 0.001
		T x Ta	7, 303.10	0.891	0.514
		T x RGT	7, 325.54	0.352	0.929
		Ta x RGT	1, 542.38	44.470	< 0.001
		C x T x Ta	7, 303.10	0.417	0.891
		C x T x RGT	7, 325.54	0.420	0.890
		C x Ta x RGT	1, 533.79	2.625	0.106
		T x Ta x RGT	7, 303.10	0.902	0.505
		C x T x Ta x RGT	7, 303.10	0.863	0.536
		Gender	1, 368.12	0.163	0.687
		Age	1, 359.70	3.643	0.057
		BMI	1, 359.18	1.013	0.315
		Baseline Glucose	1, 372.23	8.003	0.005
		Baseline Reaction Time	1, 1209.94	1018.160	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Mood:					
Alertness	3130.13	Condition (C)	1, 79.94	3.505	0.065
		Time (T)	4, 80	2.818	0.030
		Regulator Type (RGT)	1, 80.29	0.793	0.376
		C x T	4, 80	0.455	0.768
		C x RGT	1, 79.88	2.238	0.139
		T x RGT	4, 80	1.030	0.397
		C x T x RGT	4, 80	0.847	0.500
		Gender	1, 80.03	0.856	0.358
		Age	1, 80	5.751	0.019
		BMI	1, 80	9.585	0.003
		Baseline Glucose	1, 80.17	1.001	0.320
		Baseline Alertness	1, 80	27.377	< 0.001
Anxiety	3154.87	Condition (C)	1, 80.02	3.871	0.053
		Time (T)	4, 80	0.730	0.574
		Regulator Type (RGT)	1, 81.77	0.485	0.488
		C x T	4, 80	0.632	0.641
		C x RGT	1, 80.27	0.016	0.899
		T x RGT	4, 80	1.796	0.138
		C x T x RGT	4, 80	0.802	0.527
		Gender	1, 80.03	0.163	0.688
		Age	1, 80	4.640	0.034
		BMI	1, 80	0.835	0.364
		Baseline Glucose	1, 80.15	1.059	0.307
		Baseline Anxiety	1, 80	42.629	< 0.001
Contentment	2873.37	Condition (C)	1, 77.12	0.063	0.803
		Time (T)	4, 80	0.858	0.493
		Regulator Type (RGT)	1, 82.66	1.114	0.294
		C x T	4, 80	0.717	0.583
		C x RGT	1, 76.47	1.434	0.235
		T x RGT	4, 80	2.009	0.101
		C x T x RGT	4, 80	0.064	0.992
		Gender	1, 78.23	0.416	0.521
		Age	1, 79.34	4.730	0.033
		BMI	1, 78.59	0.002	0.969
		Baseline Glucose	1, 78.74	1.061	0.306
		Baseline Contentment	1, 78.96	49.829	< 0.001

Table F2: Continued.

Study 2 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
HFS data:					
Hunger	3504.51	Condition (C)	1, 80.67	0.024	0.878
		Time (T)	4, 80	1.747	0.148
		Regulator Type (RGT)	1, 92.42	0.210	0.648
		C x T	4, 80	1.069	0.377
		C x RGT	1, 79.55	0.015	0.901
		T x RGT	4, 80	3.210	0.017
		C x T x RGT	4, 80	1.304	0.276
		Gender	1, 80.03	15.401	0.000
		Age	1, 80	0.379	0.540
		BMI	1, 80	3.722	0.057
		Baseline Glucose	1, 80.16	0.077	0.782
		Baseline Hunger	1, 80	24.622	< 0.001
Fullness	3525.43	Condition (C)	1, 79.01	0.944	0.334
		Time (T)	4, 80	0.431	0.786
		Regulator Type (RGT)	1, 84.26	0.124	0.726
		C x T	4, 80	0.430	0.787
		C x RGT	1, 78.33	0.001	0.977
		T x RGT	4, 80	2.424	0.055
		C x T x RGT	4, 80	0.723	0.579
		Gender	1, 80.03	16.800	< 0.001
		Age	1, 80	0.218	0.642
		BMI	1, 80	1.958	0.166
		Baseline Glucose	1, 80.17	0.087	0.769
		Baseline Fullness	1, 80	10.842	0.001
Sleepiness	3446.23	Condition (C)	1, 79.88	0.960	0.330
		Time (T)	4, 80	2.139	0.084
		Regulator Type (RGT)	1, 82.94	0.002	0.965
		C x T	4, 80	0.665	0.618
		C x RGT	1, 79.82	0.515	0.475
		T x RGT	4, 80	1.187	0.323
		C x T x RGT	4, 80	1.579	0.188
		Gender	1, 80.02	0.747	0.390
		Age	1, 80	10.241	0.002
		BMI	1, 80	2.713	0.103
		Baseline Glucose	1, 80.10	0.428	0.515
		Baseline Sleepiness	1, 80	22.391	< 0.001

Table F3: LMM results for Study 3 (Chapter 6).

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Glucose:					
Glycaemic Response	2607.27	Condition (C)	1, 63.74	2.978	0.089
		Time (T)	21, 379.54	39.349	< 0.001
		Regulator Type (RGT)	1, 73.93	19.711	< 0.001
		C x T	21, 379.54	4.416	< 0.001
		C x RGT	1, 63.74	0.534	0.468
		T x RGT	21, 379.54	3.090	< 0.001
		C x T x RGT	21, 379.54	0.852	0.654
		Gender	1, 38.62	1.117	0.297
		Age	1, 38.62	5.436	0.025
		BMI	1, 38.62	0.231	0.634
		Baseline Glucose	1, 38.62	981.557	< 0.001
Cognition:					
CRT Accuracy	1047.36	Condition (C)	1, 46.15	0.018	0.895
		Time (T)	7, 50	1.116	0.368
		Regulator Type (RGT)	1, 59.98	0.009	0.925
		C x T	7, 50	2.060	0.065
		C x RGT	1, 46.48	0.202	0.655
		T x RGT	7, 50	0.624	0.734
		C x T x RGT	7, 50	0.266	0.964
		Gender	1, 50	0.989	0.325
		Age	1, 50	0.032	0.859
		BMI	1, 50	0.946	0.335
		Baseline Glucose	1, 50	1.930	0.171
		Baseline Accuracy	1, 50	34.730	< 0.001
CRT Reaction Time	3654.36	Condition (C)	1, 39.34	0.345	0.560
		Time (T)	7, 50	1.982	0.076
		Regulator Type (RGT)	1, 55.27	5.810	0.019
		C x T	7, 50	1.510	0.185
		C x RGT	1, 39.40	0.397	0.532
		T x RGT	7, 50	0.881	0.528
		C x T x RGT	7, 50	0.407	0.894
		Gender	1, 50	40.148	< 0.001
		Age	1, 50	15.084	< 0.001
		BMI	1, 50	8.763	0.005
		Baseline Glucose	1, 50	14.124	< 0.001
		Baseline Reaction Time	1, 50	34.987	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Cognition:					
RVIP Accuracy	1330.64	Condition (C)	1, 37.97	0.000	0.997
		Time (T)	7, 50	1.081	0.389
		Regulator Type (RGT)	1, 51.91	1.490	0.228
		C x T	7, 50	2.356	0.037
		C x RGT	1, 37.96	0.049	0.826
		T x RGT	7, 50	1.334	0.254
		C x T x RGT	7, 50	0.628	0.731
		Gender	1, 50	2.908	0.094
		Age	1, 50	0.671	0.417
		BMI	1, 50	3.731	0.059
		Baseline Glucose	1, 50	0.061	0.806
		Baseline Accuracy	1, 50	88.638	< 0.001
RVIP Reaction Time	3756.21	Condition (C)	1, 39.98	0.077	0.783
		Time (T)	7, 50	1.890	0.091
		Regulator Type (RGT)	1, 56.96	2.641	0.110
		C x T	7, 50	1.365	0.241
		C x RGT	1, 40.08	0.005	0.944
		T x RGT	7, 50	1.196	0.322
		C x T x RGT	7, 50	1.265	0.287
		Gender	1, 50	1.851	0.180
		Age	1, 50	0.547	0.463
		BMI	1, 50	10.205	0.002
		Baseline Glucose	1, 50	6.747	0.012
		Baseline Reaction Time	1, 50	83.502	< 0.001
Merged Accuracy	1884.60	Condition (C)	1, 48.51	0.058	0.811
		Time (T)	7, 50	1.549	0.173
		Regulator Type (RGT)	1, 53.81	2.945	0.092
		C x T	7, 50	0.665	0.700
		C x RGT	1, 48.39	0.026	0.873
		T x RGT	7, 50	0.919	0.500
		C x T x RGT	7, 50	1.832	0.102
		Gender	1, 50	0.104	0.748
		Age	1, 50	0.272	0.605
		BMI	1, 50	2.167	0.147
		Baseline Glucose	1, 50	0.449	0.506
		Baseline Accuracy	1, 50	129.621	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Cognition:					
Merged Reaction Time	3466.87	Condition (C)	1, 41.18	0.003	0.957
		Time (T)	7, 41.86	2.058	0.070
		Regulator Type (RGT)	1, 48.85	0.009	0.923
		C x T	7, 41.86	1.043	0.416
		C x RGT	1, 41.10	0.016	0.899
		T x RGT	7, 41.86	0.162	0.991
		C x T x RGT	7, 41.86	3.583	0.004
		Gender	1, 33.34	2.452	0.127
		Age	1, 32.78	8.349	0.007
		BMI	1, 35.52	0.001	0.981
		Baseline Glucose	1, 33.79	3.350	0.076
		Baseline Reaction Time	1, 36.61	140.802	< 0.001
LM Accuracy	1530.56	Condition (C)	1, 44.53	0.133	0.717
		Time (T)	7, 50	4.750	< 0.001
		Regulator Type (RGT)	1, 56.80	0.003	0.955
		C x T	7, 50	0.901	0.513
		C x RGT	1, 44.64	0.862	0.358
		T x RGT	7, 50	1.132	0.359
		C x T x RGT	7, 50	3.107	0.008
		Gender	1, 50	1.515	0.224
		Age	1, 49.25	3.447	0.069
		BMI	1, 48.89	1.365	0.248
		Baseline Glucose	1, 49.27	1.049	0.311
		Baseline Accuracy	1, 49.75	73.877	< 0.001
LM Reaction Time	5890.57	Condition (C)	1, 49.01	0.005	0.944
		Time (T)	7, 50	1.799	0.108
		Regulator Type (RGT)	1, 59.41	0.012	0.915
		C x T	7, 50	1.461	0.203
		C x RGT	1, 48.49	0.128	0.722
		T x RGT	7, 50	0.533	0.805
		C x T x RGT	7, 50	2.349	0.037
		Gender	1, 50	1.998	0.164
		Age	1, 50	4.421	0.041
		BMI	1, 50	0.061	0.806
		Baseline Glucose	1, 50	6.392	0.015
		Baseline Reaction Time	1, 50	115.673	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Global Cognition:					
Global Accuracy	531.12	Condition (C)	1, 48.72	0.135	0.715
		Time (T)	7, 50	2.111	0.059
		Regulator Type (RGT)	1, 5481	0.797	0.376
		C x T	7, 50	2.771	0.016
		C x RGT	1, 48.67	0.035	0.853
		T x RGT	7, 50	1.062	0.402
		C x T x RGT	7, 50	0.700	0.672
		Gender	1, 50	2.846	0.098
		Age	1, 50	1.415	0.240
		BMI	1, 50	0.936	0.338
		Baseline Glucose	1, 50	0.233	0.632
		Baseline Accuracy	1, 50	169.802	< 0.001
Global Reaction Time	483.25	Condition (C)	1, 47.95	0.206	0.652
		Time (T)	7, 50	1.867	0.095
		Regulator Type (RGT)	1, 58.39	0.230	0.633
		C x T	7, 50	2.108	0.060
		C x RGT	1, 47.67	0.133	0.717
		T x RGT	7, 50	0.824	0.572
		C x T x RGT	7, 50	0.623	0.735
		Gender	1, 50	3.472	0.068
		Age	1, 50	5.726	0.021
		BMI	1, 50	2.406	0.127
		Baseline Glucose	1, 50	2.623	0.112
		Baseline Reaction Time	1, 50	106.857	< 0.001
Global Performance	365.35	Condition (C)	1, 44.51	0.636	0.429
		Time (T)	7, 50	2.099	0.061
		Regulator Type (RGT)	1, 55.39	0.140	0.709
		C x T	7, 50	2.500	0.028
		C x RGT	1, 44.29	0.147	0.703
		T x RGT	7, 50	1.390	0.231
		C x T x RGT	7, 50	0.590	0.761
		Gender	1, 50	2.973	0.091
		Age	1, 50	2.933	0.093
		BMI	1, 50	1.147	0.289
		Baseline Glucose	1, 50	0.520	0.474
		Baseline Performance	1, 50	270.323	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Task Comparisons:					
CRT vs. Merged (Percent Correct)	4343.27	Condition (C)	1, 289.68	2.191	0.140
		Time (T)	7, 98.90	0.563	0.785
		Task (Ta)	1, 433.59	46.825	< 0.001
		Regulator Type (RGT)	1, 363.24	6.676	0.010
		C x T	7, 98.90	0.596	0.758
		C x Ta	1, 320.69	2.731	0.099
		C x RGT	1, 289.61	2.852	0.092
		T x Ta	7, 96.18	0.307	0.949
		T x RGT	7, 98.90	0.489	0.840
		Ta x RGT	1, 329.89	11.220	0.001
		C x T x Ta	7, 96.18	1.129	0.351
		C x T x RGT	7, 98.90	0.811	0.581
		C x Ta x RGT	1, 319.78	1.729	0.189
		T x Ta x RGT	7, 96.18	0.776	0.609
		C x T x Ta x RGT	7, 96.18	0.685	0.685
		Gender	1, 368.43	16.126	0.000
		Age	1, 387.54	2.277	0.132
		BMI	1, 350.20	12.269	0.001
		Baseline Glucose	1, 353.51	1.004	0.317
		Baseline Percent	1, 627.17	209.277	< 0.001
CRT vs. Merged (Reaction Time)	7604.03	Condition (C)	1, 150.38	1.100	0.296
		Time (T)	7, 237.42	0.885	0.519
		Task (Ta)	1, 650.70	62.582	< 0.001
		Regulator Type (RGT)	1, 177	2.025	0.156
		C x T	7, 237.42	0.691	0.680
		C x Ta	1, 418.32	1.471	0.226
		C x RGT	1, 149.79	0.453	0.502
		T x Ta	7, 171.55	0.863	0.537
		T x RGT	7, 237.42	0.263	0.967
		Ta x RGT	1, 450.14	22.052	< 0.001
		C x T x Ta	7, 171.55	0.366	0.921
		C x T x RGT	7, 237.42	0.201	0.985
		C x Ta x RGT	1, 420.60	5.577	0.019
		T x Ta x RGT	7, 171.55	0.465	0.859
		C x T x Ta x RGT	7, 171.55	0.716	0.658
		Gender	1, 190.11	9.577	0.002
		Age	1, 206.17	28.392	< 0.001
		BMI	1, 192.69	12.243	0.001
		Baseline Glucose	1, 190.83	2.325	0.129
		Baseline Reaction Time	1, 561.99	887.789	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Task Comparisons:					
RVIP vs. Merged (Percent Correct)	6210.87	Condition (C)	1, 137.68	0.509	0.477
		Time (T)	7, 197.66	0.742	0.637
		Task (Ta)	1, 465.01	140.333	< 0.001
		Regulator Type (RGT)	1, 196.24	5.365	0.022
		C x T	7, 197.66	0.402	0.901
		C x Ta	1, 375.35	0.918	0.339
		C x RGT	1, 137.69	0.025	0.876
		T x Ta	7, 125.99	0.268	0.965
		T x RGT	7, 197.66	0.300	0.953
		Ta x RGT	1, 375.69	4.279	0.039
		C x T x Ta	7, 125.99	0.352	0.928
		C x T x RGT	7, 197.66	0.384	0.911
		C x Ta x RGT	1, 375.35	0.005	0.941
		T x Ta x RGT	7, 125.99	0.275	0.963
		C x T x Ta x RGT	7, 125.99	0.600	0.755
		Gender	1, 285.28	12.676	< 0.001
		Age	1, 283.74	18.793	< 0.001
		BMI	1, 283.17	4.889	0.028
		Baseline Glucose	1, 305.31	0.388	0.534
		Baseline Percent	1, 719.61	256.783	< 0.001
RVIP vs. Merged (Reaction Time)	7890.71	Condition (C)	1, 293.23	0.067	0.796
		Time (T)	7, 134.07	1.406	0.208
		Task (Ta)	1, 548.66	75.198	< 0.001
		Regulator Type (RGT)	1, 296.70	0.136	0.712
		C x T	7, 134.07	0.541	0.802
		C x Ta	1, 245.09	1.127	0.290
		C x RGT	1, 293.39	0.021	0.884
		T x Ta	7, 153.19	0.323	0.943
		T x RGT	7, 134.07	0.642	0.720
		Ta x RGT	1, 250.49	2.333	0.128
		C x T x Ta	7, 153.19	0.222	0.980
		C x T x RGT	7, 134.07	1.303	0.253
		C x Ta x RGT	1, 245.35	0.073	0.787
		T x Ta x RGT	7, 153.19	0.342	0.933
		C x T x Ta x RGT	7, 153.19	0.598	0.757
		Gender	1, 302.12	15.610	< 0.001
		Age	1, 286.95	35.608	< 0.001
		BMI	1, 281.85	2.793	0.096
		Baseline Glucose	1, 304.14	0.047	0.828
		Baseline Reaction Time	1, 619.20	882.569	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
Mood:					
Alertness	1864.54	Condition (C)	1, 48.14	0.064	0.802
		Time (T)	4, 50	4.121	0.006
		Regulator Type (RGT)	1, 60.90	0.006	0.937
		C x T	4, 50	0.448	0.773
		C x RGT	1, 48.44	1.824	0.183
		T x RGT	4, 50	1.551	0.202
		C x T x RGT	4, 50	0.504	0.733
		Gender	1, 50	3.300	0.075
		Age	1, 50	11.877	0.001
		BMI	1, 50	0.201	0.655
		Baseline Glucose	1, 50	0.862	0.358
		Baseline Alertness	1, 50	118.804	< 0.001
Anxiety	1914.47	Condition (C)	1, 45.21	0.412	0.524
		Time (T)	4, 50	1.821	0.139
		Regulator Type (RGT)	1, 50.87	1.552	0.219
		C x T	4, 50	0.346	0.845
		C x RGT	1, 45.27	0.002	0.963
		T x RGT	4, 50	1.182	0.330
		C x T x RGT	4, 50	1.439	0.235
		Gender	1, 50	2.751	0.103
		Age	1, 50	1.902	0.174
		BMI	1, 50	2.621	0.112
		Baseline Glucose	1, 50	5.230	0.026
		Baseline Anxiety	1, 50	51.736	< 0.001
Contentment	1602.99	Condition (C)	1, 46.25	0.168	0.683
		Time (T)	4, 50	4.637	0.003
		Regulator Type (RGT)	1, 52.47	2.405	0.127
		C x T	4, 50	1.649	0.177
		C x RGT	1, 46.27	1.739	0.194
		T x RGT	4, 50	2.091	0.096
		C x T x RGT	4, 50	1.293	0.285
		Gender	1, 50	0.172	0.680
		Age	1, 50	2.697	0.107
		BMI	1, 50	0.057	0.812
		Baseline Glucose	1, 50	0.384	0.538
		Baseline Contentment	1, 50	156.734	< 0.001

Table F3: Continued.

Study 3 Outcome Measure	Model Fit (-2LL)	Factor	df	F statistic	p value
HFS data:					
Hunger	2060.20	Condition (C)	1, 46.12	0.656	0.422
		Time (T)	4, 50	3.016	0.026
		Regulator Type (RGT)	1, 52.98	3.938	0.052
		C x T	4, 50	2.919	0.030
		C x RGT	1, 46.10	2.970	0.092
		T x RGT	4, 50	0.173	0.951
		C x T x RGT	4, 50	2.309	0.071
		Gender	1, 49.94	0.204	0.653
		Age	1, 48.53	0.058	0.811
		BMI	1, 48.82	2.509	0.120
		Baseline Glucose	1, 49.23	8.030	0.007
		Baseline Hunger	1, 48.56	55.726	< 0.001
Fullness	2104.31	Condition (C)	1, 48.14	1.037	0.314
		Time (T)	4, 50	6.497	< 0.001
		Regulator Type (RGT)	1, 55.65	5.725	0.020
		C x T	4, 50	1.817	0.140
		C x RGT	1, 47.96	2.863	0.097
		T x RGT	4, 50	0.741	0.569
		C x T x RGT	4, 50	1.511	0.213
		Gender	1, 50	2.545	0.117
		Age	1, 50	0.199	0.657
		BMI	1, 50	1.165	0.286
		Baseline Glucose	1, 50	3.721	0.059
		Baseline Fullness	1, 50	56.380	< 0.001
Sleepiness	2105.08	Condition (C)	1, 49.63	0.165	0.686
		Time (T)	4, 50	1.964	0.114
		Regulator Type (RGT)	1, 50.91	0.404	0.528
		C x T	4, 50	2.521	0.053
		C x RGT	1, 49.75	0.695	0.409
		T x RGT	4, 50	1.353	0.264
		C x T x RGT	4, 50	0.582	0.677
		Gender	1, 50	2.189	0.145
		Age	1, 50	3.921	0.053
		BMI	1, 50	0.238	0.628
		Baseline Glucose	1, 50	0.093	0.761
		Baseline Sleepiness	1, 50	39.274	< 0.001

Appendix G – Participant Information Sheets

Appendix G1 – Study 1 (Chapter 4) Participant Information Sheet

Department of Psychology

Matthew Grout:

Whiteknights

PO Box 266, Reading RG6 6AP, UK

PARTICIPANT INFORMATION SHEET

The effect of glycaemic index variation on blood glucose and mood in people across the day (GI Study)

You are being invited to take part in a research study. Before you decide if you want to take part it is important that you understand what is involved. Please read the following information and discuss with others if you wish. Please ask us if there is anything you do not understand and if you would like any additional information. Take the time to decide whether or not you wish to take part.

Aim

The aim of this study is to evaluate the effects that a foods glycaemic index value has on blood glucose and mood individuals aged 18-65 years old. About 24 volunteers will take part in this study. Equal numbers of men and women will be included in the study cohort.

What is the Glycaemic Index (GI)?

- A value assigned to a food that represents its' rate of glucose release.
- A higher value would suggest that a food releases glucose at a faster rate.
- Values range from 0 to 100, with pure glucose having a value of 100.

Proposed effects of the Glycaemic Index?

- Research suggests that the glycaemic index of foods can have both physiological and cognitive effects.
- Long term consumption of high GI foods has been associated with higher risk of diabetes, obesity and some forms of cancer.
- Low GI foods have been associated with a slower decline in cognitive ability, as well as improved memory and attention.

Why is this study being carried out?

There are an increasing number of studies which have investigated the glycaemic index of foods and how they relate to our body and cognition. However, many of these studies are limited to a single meal, whilst the rest look at only two meals. Therefore, this study aims to measure both blood glucose levels and mood across three meals to extend our knowledge of the glycaemic index.

Inclusion criteria/Exclusion criteria

Inclusion criteria – If the following applies to you, you will be considered for participation in the trial:

- Aged between 18 and 65 years of age.
- Willing to participate in the entire study (signed informed consent required)
- Subjects will be eligible for the study if male or female (not pregnant or lactating)

Exclusion criteria – If the following applies to you, you will be unable to participate in the trial:

- Diabetic
- Smoker
- Have any food intolerances or allergies
- History of alcohol or drug misuse
- Diagnosed with any of the following:
    - High blood cholesterol
    - High blood pressure
    - Thyroid disorder
    - Heart problems, stroke or any vascular disease in the past 12 months
    - Inflammatory diseases such as rheumatoid arthritis
    - Bone related conditions, such as osteoporosis
    - Renal, gastrointestinal, respiratory, liver disease or cancer
- You are presently taking part in another clinical trial or research study
- You are an elite athlete (very high intensity training more than 3 times a week)
- You are currently on a specific diet or taking any dietary supplements and are unwilling to cease during the testing period
- You are intending to regularly use medication which affects gastrointestinal motility

What will I be asked to do?

- All participants will be asked to fill out a health screening questionnaire and inclusion/exclusion criteria will be reviewed for volunteer eligibility.
- Written informed consent from you will be required.

- On giving consent, participants will have a screening session. During this your height and weight will be measured as well as your blood pressure. Your fasting blood glucose levels will also be tested via a finger prick technique.
- Once the study begins, participants will be randomly allocated to the order in which they will complete the arms of the study. The two arms consist of a full day of consuming either 3 high GI meals or 3 low GI meals. You will not be told which arm you are taking part in on each of the visits until the end of the study.
- During each test day, the participant will be required to consume either 3 meals varying in their GI value. Throughout the day, the participant will have their mood measured six times by completing a questionnaire as well as having their blood glucose measured a total of 21 times via finger pricks.
- On completion of the first study visit, the investigator and participant will arrange the next two study visits in advance. Each study visit will be spaced 1-4 weeks apart.
- All testing will occur in the Hugh Sinclair Unit of Human Nutrition within the Department of Food and Nutritional Sciences, University of Reading.
- Any adverse medical events which occur during the trial (e.g. headache) will be recorded on an adverse event form along with any treatment required.
- Participants will be removed from the study if they develop acute gastrointestinal illness or if they do not comply with the above stated restrictions.

Any there any risks?

Blood samples will be collected by experienced staff trained for this purpose at the University (Hugh Sinclair Unit). The use of finger pricks causes minimal pain, and these is a small chance of a little discomfort but every care will be taken to minimise this.

The test meals are prepared in a controlled study kitchen by the researchers who have received food safety training.

Restrictions during testing

- Participants must not eat from 21:00 the evening before a test day.
- Participants must not eat or drink the morning of a test day.
- Participants must not consume any other food during a test day other than the meals provided.
- Participants must not exercise the day before and of testing.
- Participants must not drink alcohol the day before testing.
- Participants must comply with the above stated restrictions.

General Information

- You will receive £100 for completing the research study (£25 per study visit plus £25 for completing the study). Volunteers that drop out will have their payment pro-rated to cover the part of the study completed.
- You will be provided with breakfast on the day of screening.
- If at any time you wish to withdraw from the study you are completely free to do so without giving a reason.
- The information collected will be used for research purposes only. All information will be confidential and individuals' names will not be used in any reports resulting from this work.
- Once the study has been completed, you can request the overall results and findings.
- The University has appropriate insurance and is well used to carrying out these types of trials.
- If there is a complaint then this should be addressed to Professor Julie Lovegrove, Head of the Hugh Sinclair Unit of Human Nutrition).

The investigators thank you for taking the time to read this information sheet.

If you have any queries, please feel free to contact us:

Matthew Grout

Appendix G2 – Study 2 (Chapter 5) Participant Information Sheet

Participant Information Sheet

Researchers (principal):
Professor Julie Lovegrove, *Email*: , *Phone*:
Doctor Daniel Lamport, *Email:* , *Phone:*

Researcher (role): Mr Matthew Grout
Email: ,
Contact address: School of Life Sciences, Psychology Building, Whiteknights, Unversity of Reading, RG6 6UA

Study Title: The Effect of Glycaemic Index on Cognitive Performance, Blood Glucose and Mood across the day

Investigators: Mr Matthew Grout, Professor Julie Lovegrove, and Doctor Daniel Lamport

Contact Name: Mr Matthew Grout,

Email:

Thank you for your interest in the **Glycaemic Index study.**

Before you decide to participate, it is important that you understand why the research is being completed and what it will involve. Please take your time to read the following information carefully and discuss it with others if you wish. Please ask us if anything is not clear or if you would like further information and take your time to decide whether or not you wish to take part. Thank you for reading this information sheet.

Background

Research suggests that the glycaemic index of foods can have physiological and cognitive effects. There are an increasing number of studies which have investigated the glycaemic index of foods and how they relate to health and cognition. However, many of these studies are limited to a single meal or drink. Therefore, this study aims to measure blood sugar (glucose) levels, cognition and mood across three meals to extend our knowledge of the health effects.

What is the Glycaemic Index?

- A value assigned to a food that represents its' rate of glucose release.

- A higher value would suggest that a food releases glucose at a faster rate.
- Values range from 0 to 100, with a glucose solution having a value of 100.

Why are we doing this study?

Understanding how multiple meals affect our cognition throughout the day could have many real world applications, such as tailoring what we eat in the work place to make ourselves more productive. Understanding the role of sugar (glucose) levels and how the glycaemic index affects these across the day is also important as tailoring these correctly could reduce the risk of glucose related diseases such as diabetes.

What is the purpose of the study?

The key aims of this study are to explore the effects of glycaemic index on cognitive function, blood glucose and mood in individuals aged 18-25 years old. Approximately 40 volunteers will take part in this study with equivalent numbers of males and females.

Who would we like, is eligible, to participate in the study? Why have I been invited?

Inclusion criteria – If the following applies to you, you will be considered for participation in the trial:

- Aged between 18 and 25 years of age.
- Willing to participate in the entire study (signed informed consent required)
- Male or female (not pregnant)

Exclusion criteria – If the following applies to you, you will be unable to participate in the trial:

- Suffer from diabetes
- Are anaemic
- Smoker
- Have any food intolerances or allergies (see Foods List)
- History of alcohol or drug misuse
- Diagnosed with any of the following:
    - High blood cholesterol
    - High blood pressure
    - Thyroid disorder
    - Heart problems, stroke or any vascular disease in the past 12 months
    - Inflammatory diseases such as rheumatoid arthritis
    - Bone related conditions, such as osteoporosis
    - Renal, gastrointestinal, respiratory, liver disease or cancer
- You are presently taking part in another clinical trial or research study
- You are an elite athlete (very high intensity training more than 3 times a week)
- You are currently on a specific diet, and are unwilling to cease during the testing period

- You are intending to regularly use medication which affects gastrointestinal motility

A medical and lifestyle questionnaire will be used to screen for the above criteria. If you are interested in taking part after reading this information sheet, please contact the Hugh Sinclair Unit of Human Nutrition clinical unit manager on or email:

Study Foods List:

- All Bran Cereal
- Apple (raw)
- Apple Juice
- Cashew Nuts
- Cheese
- Corn Flakes
- Flora
- Jelly Beans
- Lettuce
- Lucozade (original)
- Pasta Bake
- Philadelphia Light Spread
- Skimmed Milk
- White Bread
- Yoghurt (lemon curd)
- Yoghurt (low fat, natural)

Do I have to take part?

It is up to you to decide whether you wish to take part, you are under no obligation to participate. We will describe all of the aspects of the study to you and what each stage of the study contains. We will also go through this information sheet, which we will then give or send to you by mail. We will then ask you to sign a consent form to show you have agreed to take part. You are however, free to withdraw from the study at any time, without giving a reason

Screening visit (one hour)

You will be invited to come for a screening visit at the Hugh Sinclair Unit of Human Nutrition in the Department of Food and Nutritional Sciences (University of Reading). The visit will take place in the morning and we would like you to arrive in an unfed state (fasted; not eating or drinking anything but water from 8 pm the night before). You can expect the following as screening:

- To have any questions you have answered.
- To sign a consent form if you wish to take part.

- Have a finger prick blood sample taken to measure glucose levels.
- Your weight, height and blood pressure will be measured.
- You will have one run through of the cognitive tasks to familiarise yourself with them.
- You will be informed of your results and told if you are eligible to take part in testing.
- If eligible, you and the researcher will agree your two test day dates.
- You will receive two standardised tests meals to take home with you (each meal is two slices of white bread and a tin of baked beans).
- Finally, you will receive a light breakfast (toast and a tea/coffee).

Study visits

For this study, you will complete two separate test days at the Hugh Sinclair Unit. Each day will follow the same procedure, with the only difference being the meals that you eat. For one day, these meals will be of low glycaemic index, and on the other day they will be of high glycaemic index. You will not be told which condition you are in. Test days must be a minimum of 7 days and a maximum of 30 days apart. Please consume your standardised meal (2 slices of white bread and baked beans) by 8pm the night before each test day. After this meal, you are asked to only consume water until testing begins the following morning.

Each test day will consist of the following:

- You arrive at the Hugh Sinclair Unit at 08:00am in a fasted/unfed state.
- A continuous glucose monitoring sensor will be applied to the back of your upper arm.
- You will have one hour to relax, while the sensor self-calibrates.
- Testing commences at 09:00am and finishes at 17:00pm.
- During this time, you will:
    - Complete 9 cognitive task batteries.
    - Complete 6 mood questionnaires.
    - Have 23 glucose scans.
    - Consume a breakfast, lunch and afternoon snack.
- At the end of a test day, the glucose sensor is removed.
- You are then free to leave the unit.

The glucose sensor has a small flexible tip that is inserted just under the skin and causes minimal, if any, discomfort. The sensor is applied by a trained researcher, who has used this equipment regularly on previous participants. During testing, you are asked to remain in the Hugh Sinclair Unit for the full study day. You will have access to drinking water, the internet and a waiting lounge in between measurements.

What will be measured in readings taken?

The readings taken through the use of the continuous glucose sensor reflect the amount of glucose in your cells. The clinical meaning of your screening results can be explained to you by your GP.

Confidentiality, storage and disposal of information

Each participant will only be identified by a random number allocated at the beginning of the study and only the researchers involved in the study will be able to link your personal data to the random number. Information obtained from the study may be published in scientific journals but only in the form of average values for the group. No results for individual subjects will be published or presented at scientific meetings. All of the procedures and tests performed in this study are being used for research purposes only and not for medical diagnosis.

Do you have to modify your diet or other activities in any way?

During the study period you will be asked not to change your diet, to exercise normally and to carry out your usual activities. However, for 24 hours before your visits, you will be advised not to drink alcohol, to avoid aerobic/intense exercise, to not eat 12 hours prior to the study visit and avoid caffeinated drinks. You will also be asked to avoid all food and drink, except water, from 8pm on the evening before you're visits. During the study period, please inform us of any newly prescribed medication that you have not mentioned in your initial screening questionnaire or if you are advised to stop any medication that you were taking at the start of the study.

Are there any benefits to taking part?

Although you will derive no individual benefit, the knowledge gained from this study will help in the understanding of the relationship between glycaemic index and cognition may shape the development of diets and studies in the future.

Are there any risks to taking part?

Finger pricks at screening may occasionally cause bruising at the site of needle penetration. Trained and experienced personnel will conduct the application of the sensor and finger pricks and clinical cover (research nurse) will be available the majority of times. A Departmental first aider will also be available in the building when screening and during the study visits. There is a minimal risk that there may be some bruising and bleeding from the site of the sensor, although this is rare.

Harm

In the event that something does go wrong and you are harmed during the study the University of Reading has in place Professional Indemnity Insurances that provide cover against negligence, error or omission for the activities of its employees.

What expenses and/or payment or equivalent be made for participation in the study?

You will be remunerated for your participation following completion of the study, which will come to a total of £100.

Will my taking part in the study be kept confidential?

No. We will inform your GP about your participation in the study only (the actual screening results will not be sent). However, if we discover any abnormalities of significance to your health we will inform both you and your GP. All information about you will be handled in confidence.

Who has reviewed the study?

This project has been reviewed by the University of Reading Research Ethics Committee and has been given a favourable opinion for conduct

Contact details for further questions, or in the event of a complaint

If you have a concern regarding any aspect of this study, you should ask to speak to the investigators who will do their best to answer your questions (see contact details below). If you remain unhappy and wish to complain formally, you can do this through the study Principal Investigator (Professor Jon Gibbins and Professor Julie Lovegrove).

Investigators and Contact details

Investigators:
Doctor Daniel Lamport
Professor J.A. Lovegrove*

Hugh Sinclair Unit, School of Chemistry, Pharmacy and Food, Whiteknights, University of Reading

* Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences, Whiteknights, University of Reading

Contact details: Professor J.A. Lovegrove Tel:
Email:

Doctor D. Lamport Tel:
Email:

Thank you for your help.

Appendix G3 – Study 3 (Chapter 6) Participant Information Sheet

Participant Information Sheet

***Researchers (principal)*:**
Professor Julie Lovegrove, *Email*: , *Phone*:
Doctor Daniel Lamport, *Email:* , *Phone:*

***Researcher (role)*:** Mr Matthew Grout
Email: ,
Contact address: School of Life Sciences, Psychology Building, Whiteknights, Unversity of Reading, RG6 6UA

Study Title: The Effect of Glycaemic Index on Cognitive Performance, Blood Glucose and Mood across the day

Investigators: Mr Matthew Grout, Professor Julie Lovegrove, and Doctor Daniel Lamport

Contact Name: Mr Matthew Grout, Email:

Thank you for your interest in the **Glycaemic Index study.**

Before you decide to participate, it is important that you understand why the research is being completed and what it will involve. Please take your time to read the following information carefully and discuss it with others if you wish. If anything is not clear, please ask the Mr Grout for further information. Thank you for reading this information sheet.

What is the purpose of the study?

The key aims of this study are to explore the effects of glycaemic index on cognitive function, blood glucose and mood in individuals aged 40-70 years old. Approximately 25 volunteers will take part in this study with equivalent numbers of males and females.

What is the Glycaemic Index?

- A value assigned to a food that represents its' rate of glucose release.
- A higher value would suggest that a food releases glucose at a faster rate.
- Values range from 0 to 100, with a glucose solution having a value of 100.

Why are we doing this study?

Understanding how multiple meals affect our cognition throughout the day could have many real world applications, such as tailoring what we eat in the work place to make ourselves more productive.

Who is eligible to participate in the study?

Inclusion criteria – If the following applies to you, you will be considered for participation in the trial:

- Aged between 40 and 70 years of age.
- Willing to participate in the entire study (signed informed consent required)
- Male or female (not pregnant)
- You currently have non-insulin dependent type 2 diabetes mellitus

Exclusion criteria – If the following applies to you, you will be unable to participate in the trial:

- Presence of any self-diagnosed and/or medically diagnosed food intolerances or allergies to foods in this study (see list below).
- Being an elite athlete (very intense exercise more than 3 times a week).
- A history of drug or alcohol abuse.
- Presence of cancer.
- Presence of clinically diagnosed depression.
- Smoker.
- Pregnancy.

Study Foods List:

- All Bran Cereal
- Apple (raw)
- Apple Juice
- Cashew Nuts
- Cheese
- Corn Flakes
- Flora
- Jelly Beans
- Lettuce
- Lucozade (original)
- Pasta Bake
- Philadelphia Light Spread
- Skimmed Milk
- White Bread
- Yoghurt (lemon curd)
- Yoghurt (low fat, natural)

A medical and lifestyle questionnaire will be used to screen for the above criteria. If you are interested in taking part after reading this information sheet, please contact Matthew Grout on

Do I have to take part?

You are under no obligation to participate. All study aspects will be fully explained by the researcher (Mr Grout). We will cover all information in this sheet at screening and answer any questions you may have. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw from the study at any time, without giving a reason.

What will be involved if you take part?

Initially, you will be asked to complete a medical and lifestyle questionnaire. The researcher will examine your answers to check your eligibility. If eligible, you will be invited for a screening session.

Screening visit (one hour)

You will be invited to come for a screening visit at the Hugh Sinclair Unit of Human Nutrition in the Department of Food and Nutritional Sciences (University of Reading). The visit will take place in the morning and we would like you to arrive in an unfed state (fasted; not eating or drinking anything but water from 8 pm the night before). You can expect the following as screening:

- To have any questions you have answered.
- To sign a consent form if you wish to take part.
- Have a finger prick blood sample taken to measure glucose levels.
- Your weight, height and blood pressure will be measured.
- You will have one run through of the cognitive tasks to familiarise yourself with them.
- You will be informed of your results and told if you are eligible to take part in testing.
- If eligible, you and the researcher will agree your two test day dates.
- You will receive two standardised tests meals to take home with you (each meal is two slices of white bread and a tin of baked beans).
- Finally, you will receive a light breakfast (toast and a tea/coffee).

Study visits

For this study, you will complete two separate test days at the Hugh Sinclair Unit. Each day will follow the same procedure, with the only difference being the meals that you eat. For one day, these meals will be of low glycaemic index, and on the other day they will be of high glycaemic index. You will not be told which condition you are in. Test days must be a minimum of 7 days and a maximum of 30 days apart. Please consume your standardised meal (2 slices of white

bread and baked beans) by 8pm the night before each test day. After this meal, you are asked to only consume water until testing begins the following morning.

Each test day will consist of the following:

- You arrive at the Hugh Sinclair Unit at 08:00am in a fasted/unfed state.
- A continuous glucose monitoring sensor will be applied to the back of your upper arm.
- You will have one hour to relax, while the sensor self-calibrates.
- Testing commences at 09:00am and finishes at 17:00pm.
- During this time, you will:
    - Complete 9 cognitive task batteries.
    - Complete 6 mood questionnaires.
    - Have 23 glucose scans.
    - Consume a breakfast, lunch and afternoon snack.
- At the end of a test day, the glucose sensor is removed.
- You are then free to leave the unit.

The glucose sensor has a small flexible tip that is inserted just under the skin and causes minimal, if any, discomfort. The sensor is applied by a trained researcher, who has used this equipment regularly on previous participants. During testing, you are asked to remain in the Hugh Sinclair Unit for the full study day. You will have access to drinking water, the internet and a waiting lounge in between measurements.

What will be measured in readings taken?

The readings taken through the use of the continuous glucose sensor reflect the amount of glucose in your cells. The clinical meaning of your screening results can be explained to you by your GP.

Confidentiality, storage and disposal of information

Your contact information and any identifiable data will be kept safe on two password protected computers. One will be managed by Mr Grout, and the other will be kept by the Hugh Sinclair Unit management team.

You will be assigned a participant number, which will be used in all data files. It is impossible for anyone to identify you from this number apart from the researchers. Although results from this study may be present in the form of scientific articles or conference presentations, no individual data will be shown. All of the procedures and tests performed in this study are being used for research purposes only and not for medical diagnosis.

All data will be kept by the principal investigator (Dr Daniel Lamport) for a period of five years once the study has been completed. After this period the data will be destroyed and will no longer exist.

Do you have to modify your diet or other activities in any way?

You will be asked:

- You are asked not to exercise or drink alcohol the day before a study visit.
- To consume the provided standardised meal the evening prior to a study visit by 8pm.
- To only consume water after eating this meal.
- To inform us of any newly prescribed medication during the study period.

Are there any benefits to taking part?

Although you will derive no individual benefit, the knowledge gained from this study will help in the understanding of the relationship between glycaemic index and cognition may shape the development of diets and studies in the future.

Are there any risks to taking part?

Finger pricks at screening may occasionally cause bruising at the site of needle penetration. Trained and experienced personnel will conduct the application of the sensor and finger pricks and clinical cover (research nurse) will be available the majority of times. A Departmental first aider will also be available in the building when screening and during the study visits. There is a minimal risk that there may be some bruising and bleeding from the site of the sensor, although this is rare.

Harm

In the event that something does go wrong and you are harmed during the study the University of Reading has in place Professional Indemnity Insurances that provide cover against negligence, error or omission for the activities of its employees.

What expenses and/or payment or equivalent be made for participation in the study?

You will receive £100 in cash from Mr Grout. This will be given to you at the end of your final test day.

Will my taking part in the study be kept confidential?

Yes. However, if we discover any abnormalities of significance to your health, we will inform both you and your GP. All information about you will be handled in confidence.

Who has reviewed the study?

The East of Scotland Research Ethics Service REC1, which has responsibility for scrutinising all proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of research ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by

monitors from University of Reading and NHS England, whose role is to check that research is properly conducted and the interests of those taking part are adequately protected.

This project has been also reviewed by the University of Reading Research Ethics Committee and has been given a favourable opinion for conduct.

Contact details for further questions, or in the event of a complaint

Any concern or complaint can be made in two ways:

- Contact one of the investigators (see end of document for contact details).
- Through the NHS complaints process (see below).

The NHS complaints procedure

For the full NHS complaints procedure please enter the following link into your internet browser address bar:

https://www.nhs.uk/nhsengland/complaints-and-feedback/pages/nhs-complaints.aspx

In the first instance, please make your complaint known via email or letter to one of the investigators (contact details below).

Study Investigators and Contact details

Investigators:

Matthew Grout[1]
Doctor Daniel Lamport[1]
Professor J.A. Lovegrove*

[1]School of Psychology and Clinical Language Sciences, Whiteknights, University of Reading

* Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences, Whiteknights, University of Reading

Contact details:

Matthew Grout Tel: N/A

Email:

Professor J.A. Lovegrove Tel:
Email:

Doctor D. Lamport Tel:

Email:

Thank you for your help.

Appendix H – Medical Health and Lifestyle Questionnaire (Chapter 4-6)

Medical and Lifestyle Questionnaire

Name:		Title:
Address:		Date of Birth: Age:
Daytime Telephone:	Evening Telephone:	Best time to call:
Weight (kg):	Height (m):	BMI (kg/m^2):
E-mail: Do you use emails on a regular basis? YES/NO		

How did you hear about the study? _____

Please circle as appropriate

<u>Medical questions</u>

1. Have you been diagnosed as having any of the following?

a) High blood pressure YES/NO

b) Diabetes or other endocrine disorders YES/NO

c) Heart problems, stroke or any vascular disease in the past 12 months YES/NO

d) Cancer
YES/NO

e) Mental health issues, such as depression
YES/NO

If 'YES' to any of the above, please give details

2. Have you been diagnosed as suffering from any other illness?
   YES/NO
   If 'YES', please give details

3. Within the past 3 months, have you taken any medication (prescription or non-prescription)?
   YES/NO
   If 'YES', what are they and for what reasons?

4. Have you had any surgery within the past 3 months or do you have surgery planned? YES/NO
   If 'YES', please give details

5. Have you ever suffered from a pulmonary embolism, deep vein thrombosis, blood clots or had a blood transfusion?
   YES/NO
   If 'YES', please give details

6. Do you have a pacemaker?
   YES/NO

7. This question is **only to female** participants.

   a) Are you pregnant?
      YES/NO

<u>Lifestyle questions</u>

8. Are you currently taking part in or within the last 3 months been involved in a clinical trial or a research study? YES/NO
   If 'YES', please give details:

9. Have you been screened or contacted recently about a study? YES/NO

If 'YES', please give details

10. Do you have any food allergies (e.g. gluten or dairy) or intolerances (e.g. lactose)? YES/NO
    If 'YES', what are they?

11. Do you use any of the following:
    a) Dietary supplements, e.g. fish oils, evening primrose oil, vitamins or minerals (such as iron or calcium); YES/NO
    b) Probiotics, e.g. Actimel, Yakult, Activia yoghurts or capsules; YES/NO
    c) Cholesterol-lowering products, e.g. Flora Pro-Activ or Benecol? YES/NO
    If 'YES' to any, please give details

12. Are you vegetarian or vegan? YES/NO
    If 'YES', please specify

13. Do you exercise more than three times a week, including walking?
    YES/NO
    If 'YES', please specify the type of exercise, frequency and intensity

14. Do you smoke?
    YES/NO
    If 'YES, please give details

15. Do you have a history of alcohol or drug misuse?
    YES/NO
    If 'YES, please give details

This is the end of the questionnaire - thank you for your time.

All information provided will remain confidential at all times.

Appendix I – Informed Consent Forms

Appendix I1 – Informed Consent Form for Study 1 (Chapter 4)

Hugh Sinclair Unit of Human Nutrition
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading, RG6 6AP

Phone +

Principle investigator: Dr Daniel Lamport

Consent Form for Glycemic Index and Cognition Study

Please initial boxes

1. I confirm that I have read and understand the Participant Information Sheet dated _____for the above study, which was explained by _____. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I authorise the Investigator to inform my General Practitioner of my participation in the study. ☐
4. I have received a copy of this Consent Form and of the accompanying Participant Information Sheet. ☐
5. I consent to an initial blood sample being taken for screening purposes, followed by a series of blood samples via fingerprick throughout the study at the times indicated on the accompanying Participant Information Sheet. ☐
6. I have had explained to me that consent for my contact details and personal information to be added to the Hugh Sinclair Unit of Human Nutrition Volunteer Database is entirely voluntary.
   Accordingly I consent as indicated below: ☐

- I consent to my contact details being stored on the Nutrition Unit Volunteer Database. ☐ Yes ☐ No
- I consent to my screening information (including date of birth, height, weight, blood pressure, smoking status, long-term use of medication, and blood test results, such as level of cholesterol, triacylglycerol, and glucose) being stored on the Nutrition Unit Volunteer Database. ☐ Yes ☐ No

- I wish to receive a summary of the overall results once the study is complete and analysed statistically.

☐ Yes ☐ No

Participant details

Name of Participant: _____ Date of Birth: _____

Signature: _____ Date: _____

Address of Participant:

(*Please add if you wish to receive the overall results of the study, and/or you consent to be part of the Hugh Sinclair Unit of Human Nutrition Volunteer Database*)

_____

_____

Telephone number: _____

General Practitioner (GP) details

Name: _____

Address: _____

_____

Telephone: _____

Witnessed by

Name of researcher taking consent: _____

Signature: _____ Date: _____

Appendix I2 – Informed Consent Form for Study 2 and 3 (Chapter 5-6)

Principle investigator: Dr Daniel Lamport

Hugh Sinclair Unit of Human Nutrition
Department of Food and Nutritional Sciences
University of Reading
PO Box 226
Reading RG6 6AP

Phone

Consent Form for Glycemic Index and Cognition Study

Please initial boxes

1. I confirm that I have read and understand the Participant Information Sheet dated _____for the above study, which was explained by _____. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I authorise the Investigator to inform my General Practitioner of my participation in the study. ☐
4. I have received a copy of this Consent Form and of the accompanying Participant Information Sheet. ☐
5. I consent to an initial fingerprick glucose reading being taken for screening purposes, followed by continuous glucose monitor readings throughout the study at the times indicated on the accompanying Participant Information Sheet. ☐
6. I have had explained to me that consent for my contact details and personal information to be added to the Hugh Sinclair Unit of Human Nutrition Volunteer Database is entirely voluntary.
   Accordingly I consent as indicated below: ☐

- I consent to my contact details being stored on the Nutrition Unit Volunteer Database. ☐ Yes ☐ No
- I consent to my screening information (including date of birth, height, weight, blood pressure, smoking status, long-term use of medication, and blood test results, such as level of cholesterol, triacylglycerol, and glucose) being stored on the Nutrition Unit Volunteer Database. ☐ Yes ☐ No

☐ Yes ☐ No

- I wish to receive a summary of the overall results once the study is complete and analysed statistically.

Participant details

Name of Participant: _____ Date of Birth: _____

Signature: _____ Date: _____

Address of Participant:

(*Please add if you wish to receive the overall results of the study, and/or you consent to be part of the Hugh Sinclair Unit of Human Nutrition Volunteer Database*)

_____

_____

Telephone number: _____

General Practitioner (GP) details

Name: _____

Address: _____

_____

Telephone: _____

Witnessed by

Name of researcher taking consent: _____

Signature: _____ Date: _____

Appendix J – Screening Session Form (Chapter 4-6)

Participant Screening Sheet

Participant:

ID:

Measure	Value
Height	
Weight	
BMI	
Blood Pressure	
Blood Glucose	

Appendix K – Ethical Approval from SREC for Study 1 (Chapter 4)

From: Peter Cooper [mailto:]
Sent: 13 May 2016 17:11
To: PCLS Ethics
Subject: RE: ethics amendment 2016-032-DL

Dear Louise,

I have reviewed the documents and am happy for the study as specified to proceed.

Regards

Peter

Appendix L – Ethical Approvals for Study 2 (Chapter 5)

Appendix L1 – Ethical Approval from SREC for Study 2

From: Anastasia Christakou [mailto:]
Sent: 15 December 2016 15:57
To: PCLS Ethics
Cc: Dan Lamport
Subject: Re: FW: 2016-206-DL - if possible please review before UREC deadline 16th Dec

The above application has SREC approval to proceed to UREC.

Anastasia

Dr Anastasia Christakou
Associate Professor in Cognitive Neurobiology
Centre for Integrative Neuroscience & Neurodynamics
School of Psychology & Clinical Language Sciences
University of Reading

Appendix L2 – Ethical Approval from UREC for Study 2

Coordinator for Quality Assurance in Research
Dr Mike Proven, BSc(Hons), PhD
Academic and Governance Services
Whiteknights House
Whiteknights, PO Box 217
Reading RG6 6AH
phone
fax
email

22 March 2017

Dear Daniel

UREC 17/06: Glycaemic index and glycaemic response: exploring an optimum 24-hour profile for cognitive function. *Favourable opinion*

Thank you for the response (your email, dated 08 February 2017, refers) addressing the issues raised by the UREC Sub-committee at its January 2017 meeting (*my Provisional Opinion email of 18 January 2017 including attachments refers*). On the basis of these responses, I can confirm that the Chair is pleased to confirm a favourable ethical opinion.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here:

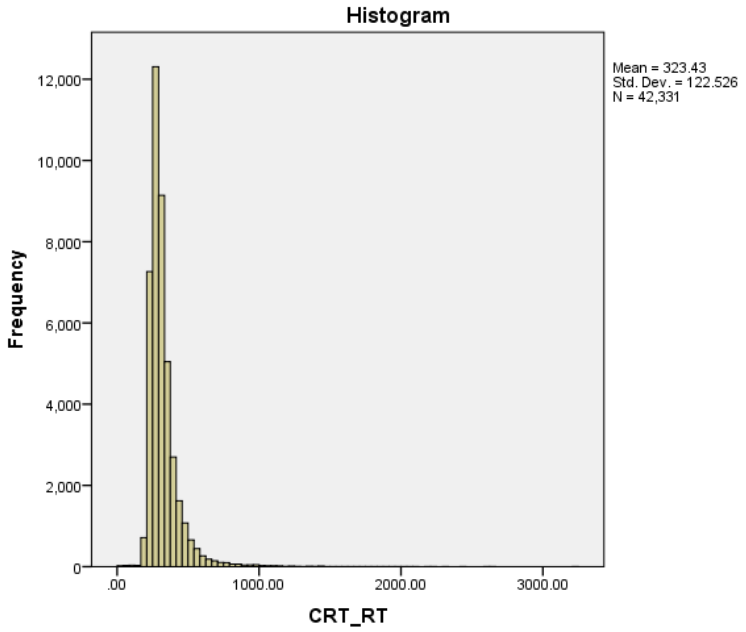
http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely
Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)

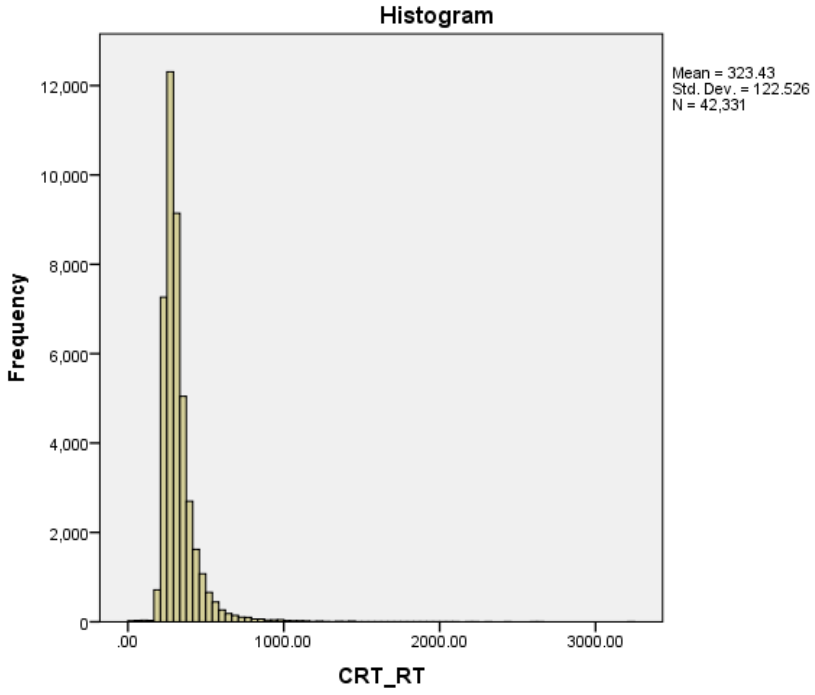
Appendix M – Cognitive outlier procedures for Study 2 (Chapter 5)

Appendix M1 – Outlier procedure for Study 2 CRT task reaction time

1. Original Histogram.

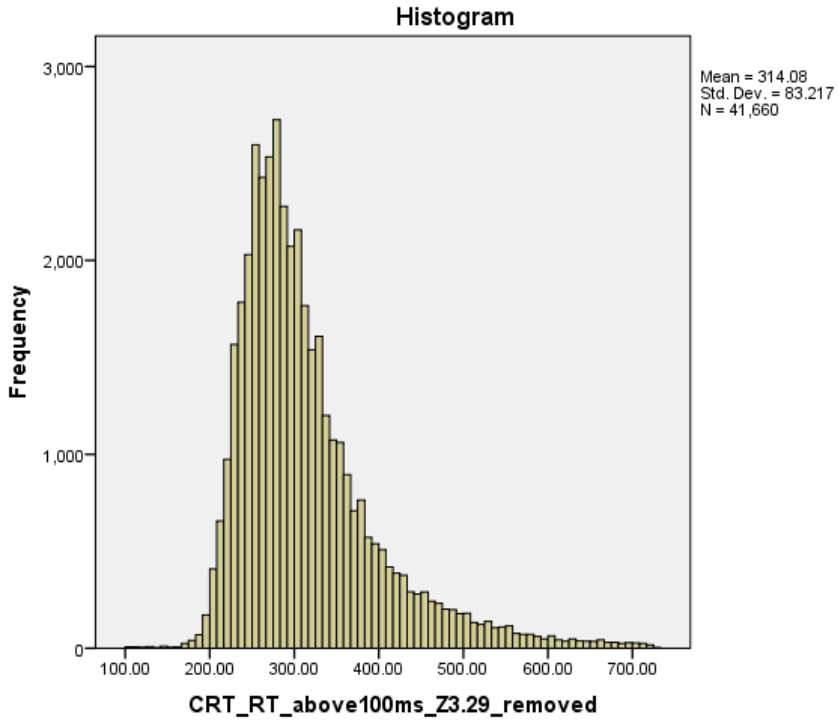


2. Histogram after removing 68 reaction times below 100ms.



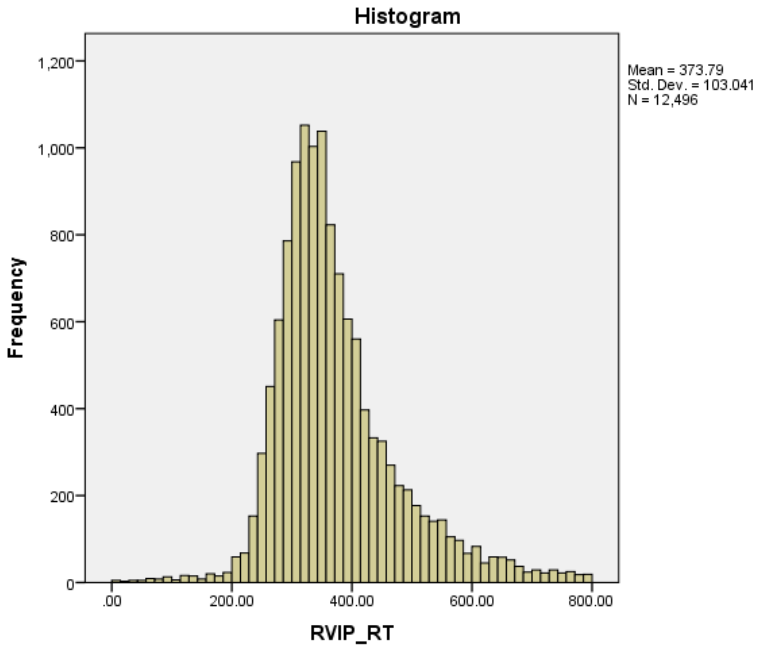
Appendix M1 – Continued.

3. Histogram after removing 603 reaction times with a Z score equal to or above 3.29.

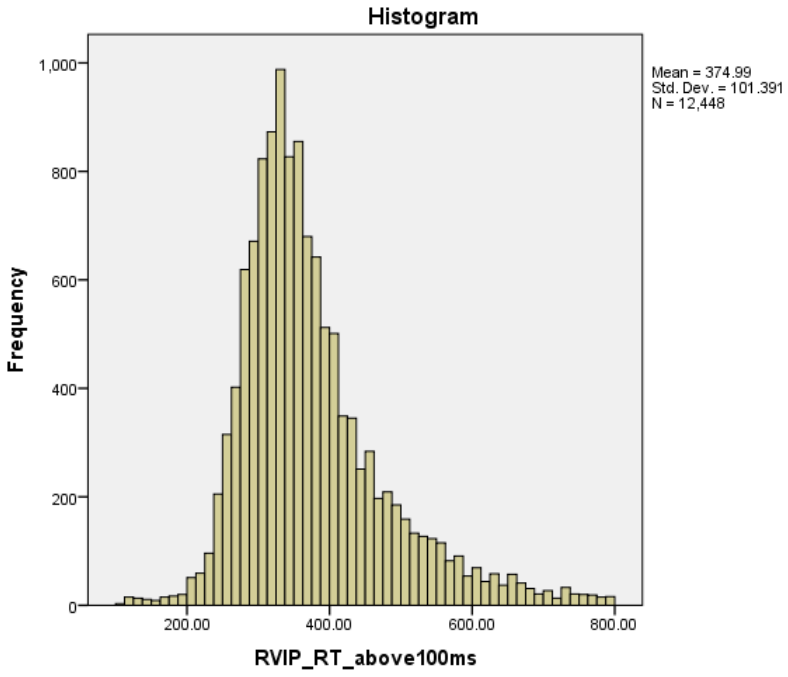


Appendix M2 – Outlier procedure for Study 2 RVIP task reaction time

1. Original Histogram.



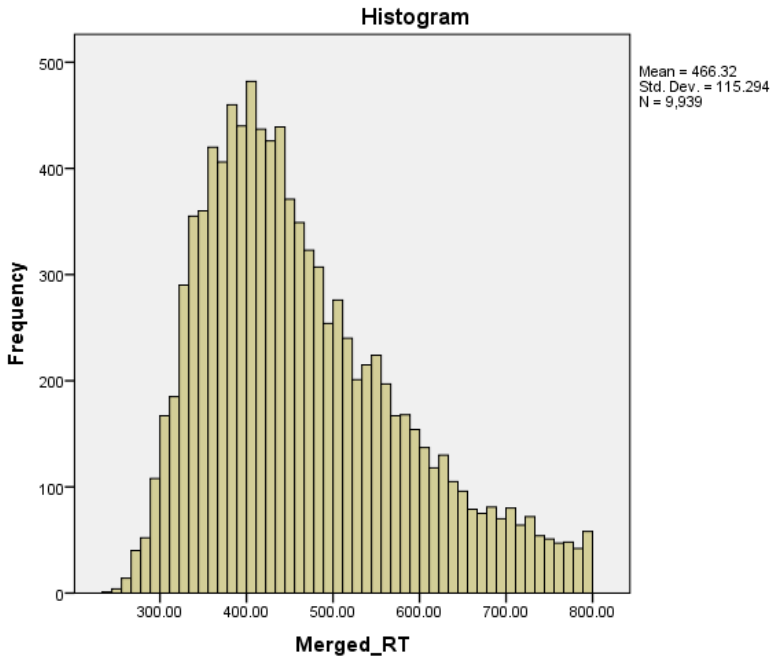
2. Histogram after removing 48 reaction times below 100ms.



3. 148 reaction times with a Z score equal to or above 3.29 were not removed (see Chapter 5).

Appendix M3 – Outlier procedure for Study 2 Merged task reaction time

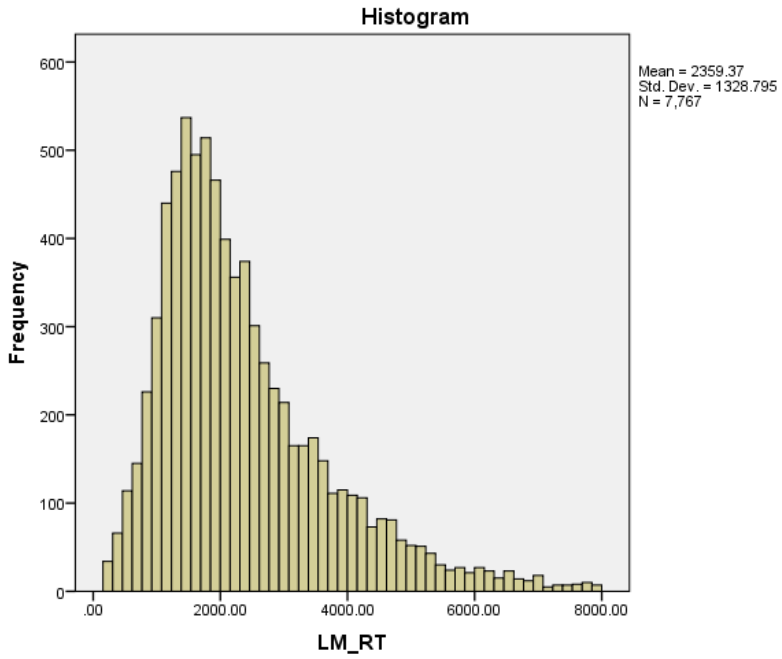
1. Original Histogram.



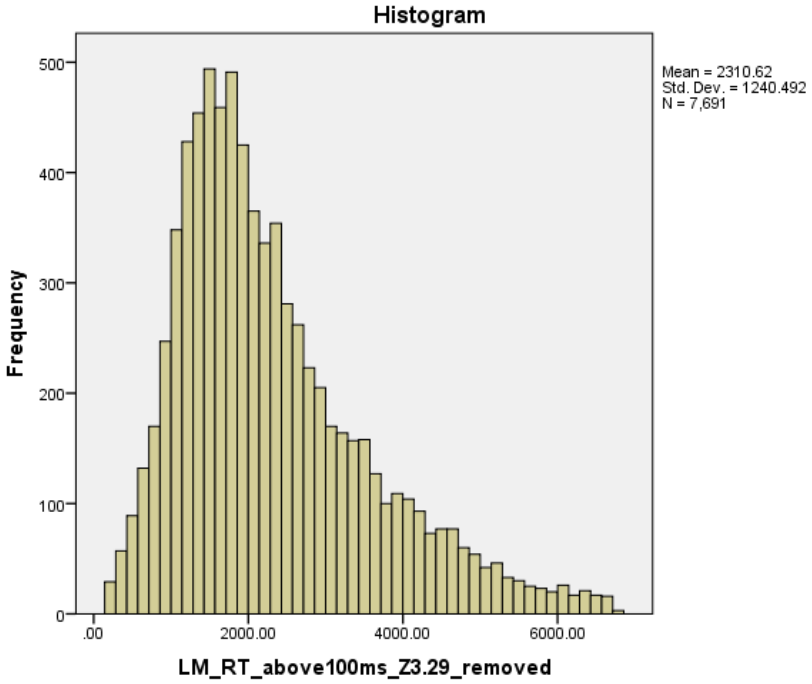
2. No reaction times were below 100ms.
3. No reaction times had a Z score equal to or above 3.29.

Appendix M4 – Outlier procedure for Study 2 LM task reaction time

1. Original Histogram.



2. No reaction times were below 100ms.
3. Histogram after removing 76 reaction times with a Z score equal to or above 3.29.



Appendix N – Ethical Approvals for Study 3 (Chapter 6)

Appendix N1 – Ethical Approval from SREC for Study 3

From: Anastasia Christakou [ ]
Sent: 14 November 2017 20:44
To: PCLS Ethics; Daniel Lamport
Subject: Re: FW: Ethics app for PhD research Lamport & Grout with UREC doc 2017-151-DL

Hi Dan,

This has SREC approval to go to UREC, but note I made some edits to the UREC form (as indicated in the attached word commented version).

If you are happy, submit but PLEASE COMPLETE THE CHECKLIST first.

If not, please edit along the lines indicated and send back to me for a signed version.

Thanks,
Anastasia

Dr Anastasia Christakou
Associate Professor in Cognitive Neurobiology
Research Ethics Chair
School of Psychology & Clinical Language Sciences
Centre for Integrative Neuroscience & Neurodynamics
University of Reading
anastasia.christakou.org

Associate Editor
Royal Society Open Science
rsos.royalsocietypublishing.org

Appendix N2 – Ethical Approval from UREC for Study 3

Coordinator for Quality Assurance in Research
Dr Mike Proven, BSc(Hons), PhD
Academic and Governance Services
Whiteknights House
Whiteknights, PO Box 217
Reading RG6 6AH
phone
fax
email

16 March 2018

Dear Daniel

UREC 17/63: Glycaemic index and glycaemic response: exploring an optimum 24-hour profile for cognitive function in type 2 diabetes. *Favourable opinion*

Thank you for the response (your email, dated 14 March 2018, email from Matthew Grout refers) addressing the issues raised by the UREC Sub-committee at its December 2017 meeting (*my Provisional Opinion email of 29 January including attachments refers*). On the basis of these responses, I can confirm that the Chair is pleased to confirm a favourable ethical opinion.

Please note that the Committee will monitor the progress of projects to which it has given favourable ethical opinion approximately one year after such agreement, and then on a regular basis until its completion.

Please also find attached Safety Note 59: Incident Reporting in Human Interventional Studies at the University of Reading, to be followed should there be an incident arising from the conduct of this research.

The University Board for Research and Innovation has also asked that recipients of favourable ethical opinions from UREC be reminded of the provisions of the University Code of Good Practice in Research. A copy is attached and further information may be obtained here:

http://www.reading.ac.uk/internal/res/QualityAssuranceInResearch/reas-RSqar.aspx.

Yours sincerely
Dr M J Proven
Coordinator for Quality Assurance in Research (UREC Secretary)

Appendix N3 – Ethical Approval from EoSRES for Study 3

From: Maeve IP Groot Bluemink [ ]
Sent: 29 March 2018 11:14
To: Matthew Grout; Daniel Lamport
Subject: 237190; 18/ES/0010 – Outcome of Application for HRA Approval

Dear Mr Grout & Dr Lamport

RE: IRAS 237190. Glycaemic Response and Cognitive Performance in diabetics (1)

Please find attached a letter informing you of the favourable outcome of your application for HRA Approval.

Please read the attached documents with care.

You may now commence your study at those participating NHS organisations in England that have confirmed their capacity and capability to undertake their role in your study (where applicable). Detail on what form this confirmation should take, including when it may be assumed, is given in Appendix B of the HRA Approval letter.

If you have any queries please do not hesitate to contact me.

Kind regards

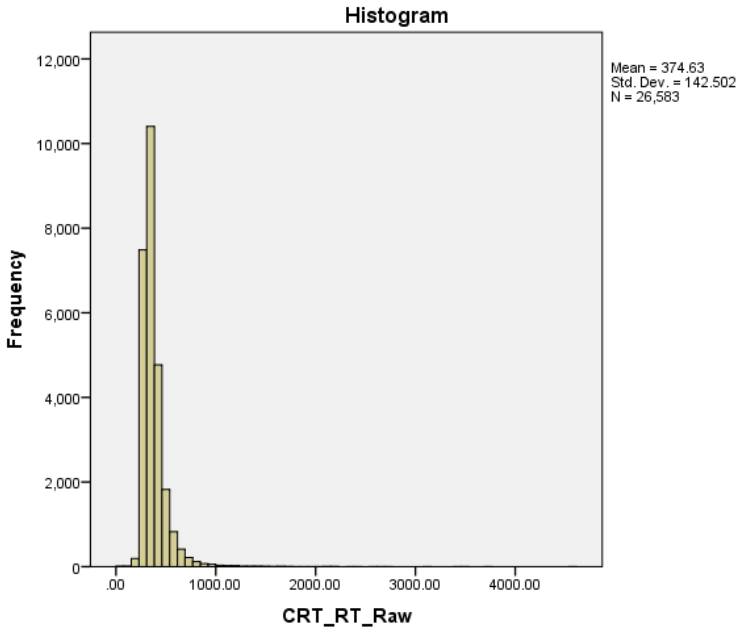
Maeve

Maeve Ip Groot Bluemink
Assessor
Health Research Authority
Bristol HRA Centre | Level 3, Block B, Whitefriars | Bristol | BS1 2NT
T.
E.
W. www.hra.nhs.uk

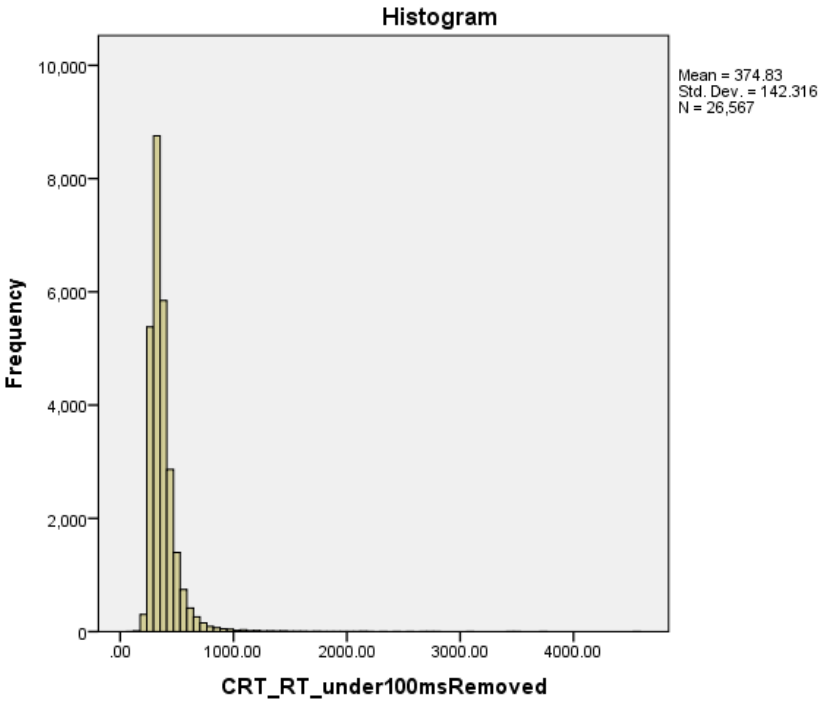
Appendix O – Cognitive outlier procedures for Study 3 (Chapter 6)

Appendix O1 – Outlier procedure for Study 3 CRT task reaction time

1. Original Histogram.

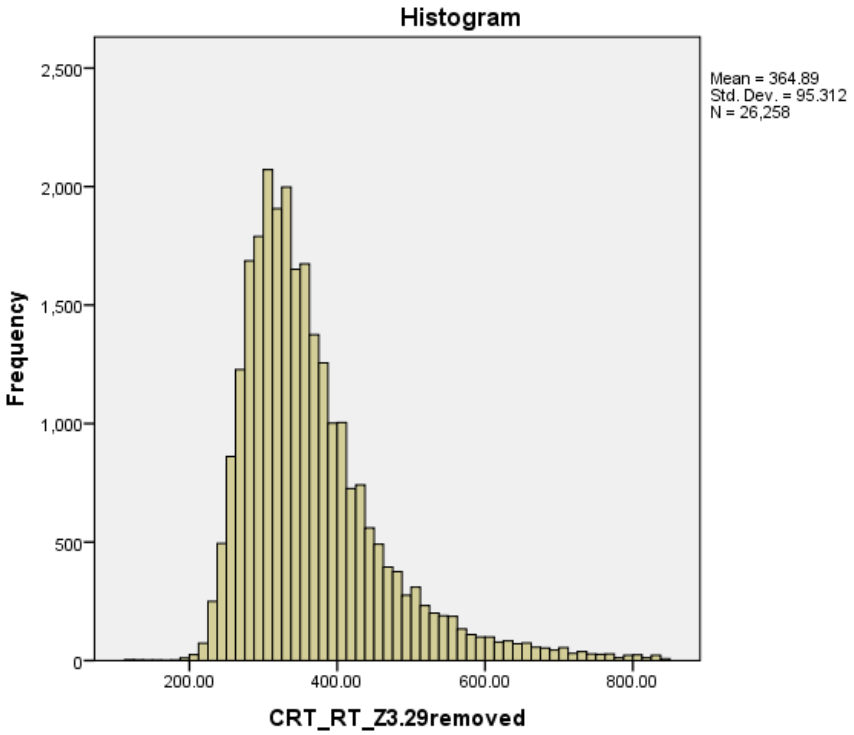


2. Histogram after removing 16 reaction times below 100ms.



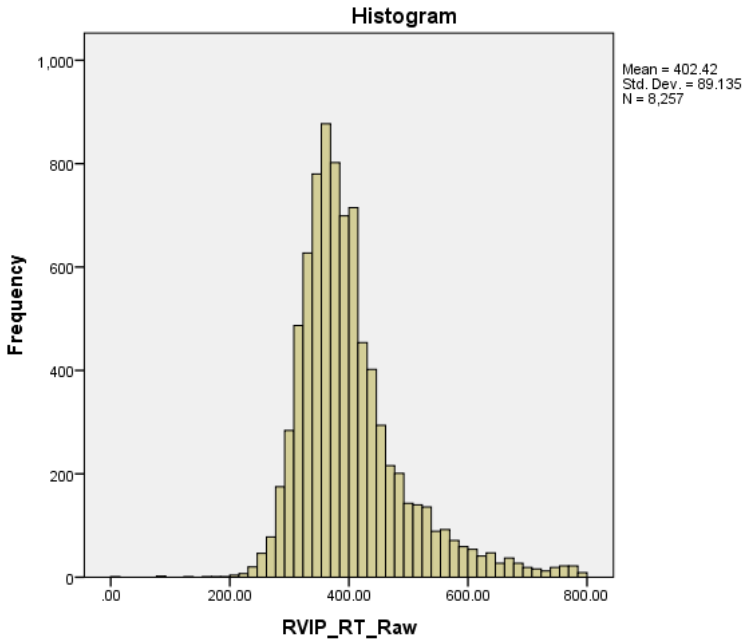
Appendix O1 – Continued.

3. Histogram after removing 309 reaction times with a Z score equal to or above 3.29.

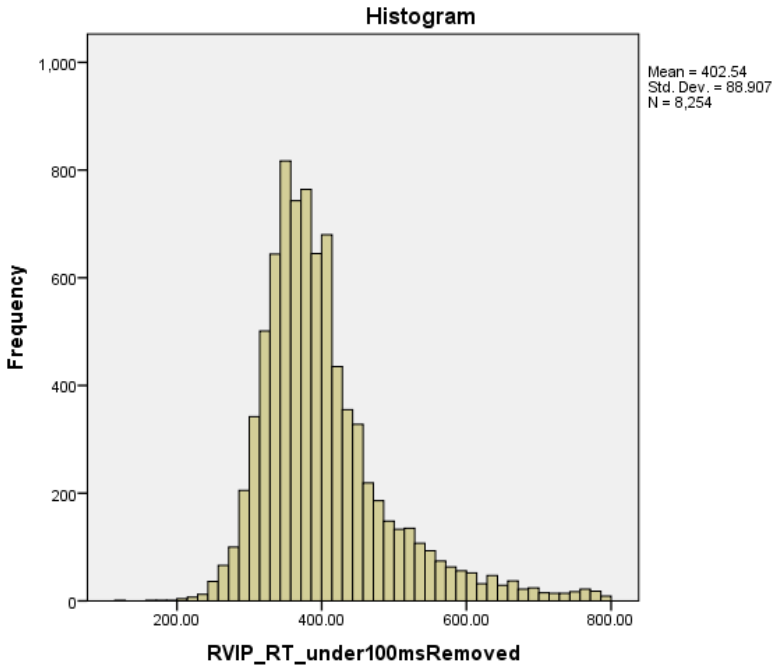


Appendix O2 – Outlier procedure for Study 3 RVIP task reaction time

1. Original Histogram.



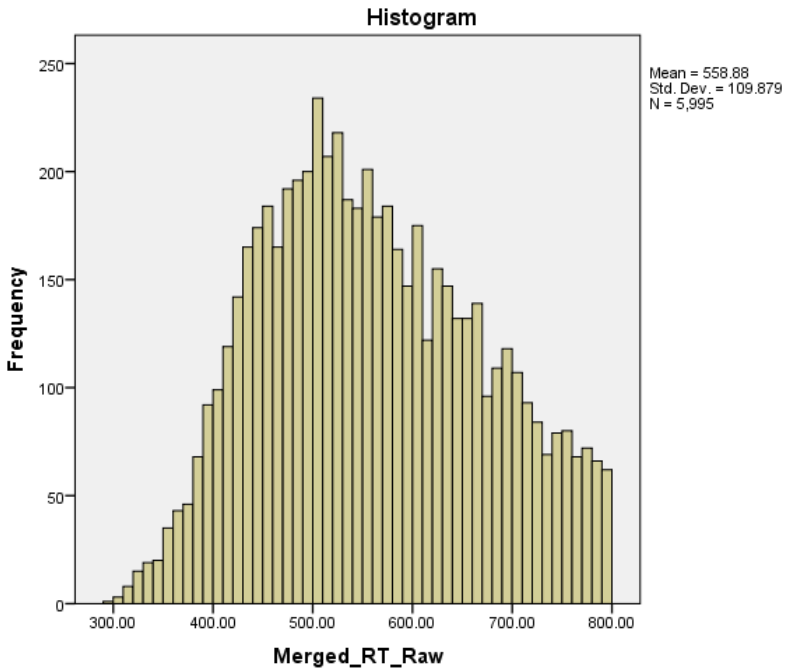
2. Histogram after removing 3 reaction times below 100ms.



3. 115 reaction times with a Z score equal to or above 3.29 were not removed (see Chapter 6).

Appendix O3 – Outlier procedure for Study 3 Merged task reaction time

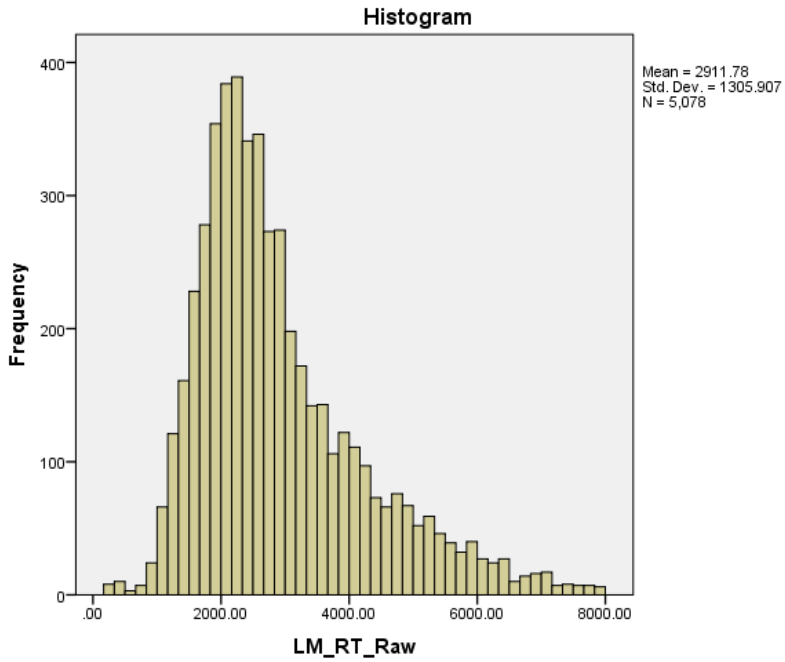
1. Original Histogram.



2. No reaction times were below 100ms.
3. No reaction times had a Z score equal to or above 3.29.

Appendix O4 – Outlier procedure for Study 3 LM task reaction time

1. Original Histogram.



2. No reaction times were below 100ms.
3. Histogram after removing 35 reaction times with a Z score equal to or above 3.29.