

Postprandial Glycaemia and Cognitive Function

Olivia Marchand

A thesis submitted in partial fulfilment of the requirements for the degree of
Master of Dietetics

At the University of Otago, Dunedin, New Zealand

June 2017

Abstract

Background: Previous research on the glycaemic index has suggested that low glycaemic index foods may have a positive effect on cognitive function. However, the body of literature on this topic has presented varying and contradicting results. This justifies further investigation of the relationship between glycaemic index and cognitive function.

Objective: To determine the effects of glycaemic index on cognitive function by examining cognition after consumption of foods that differ only by the rate of digestion of glucose in a New Zealand population of young adults.

Design: Double blinded, randomised, crossover, controlled trial.

Methods: Sixty-five participants received a higher GI trifle sweetened with sucrose and lower GI trifle sweetened with isomaltulose on separate occasions. A battery of cognitive tests was completed prior to trifle consumption, and 60, and 120 minutes after. Fingerprint blood samples were taken coincident with the cognitive tests for the determination of blood glucose concentration.

Results: There was no between-trifle difference at 60 minutes in performance on free word recall 0.0 (-0.6, 0.5), short delay word recall 0.0 (-0.5, 0.5), long delay word recall 0.0 (-0.6, 0.6), letter number sequence recall 0.3 (-0.2, 0.7) and visuo-spatial recall -0.2 (-0.6, 0.2) tests. At 120 minutes, no difference was detected in any of these tests. The participants performed 7.7 (14.9, 0.5) seconds faster in Reitan's trail test B 60 minutes after the higher GI trifle than the lower GI trifle ($P=0.037$).

Conclusion: The postprandial response to the glycaemic index of the test foods had no influence on memory. Performance on a task that combined multiple cognitive

processes may be positively influenced by higher glycaemic foods 60 minutes after intake.

Keywords: Glyc(a)emic Index, Cognitive Function, Memory, Isomaltulose, Palatinose, Glucose.

Preface

Olivia Marchand (candidate) conducted this study as part of the Master's of Dietetic degree under the supervision of Dr. Bernard Venn and Dr. Charlene Rapsey. Dr. Bernard Venn was responsible for formulating the study topic and design, gaining ethical approval and supervising the thesis write-up. Dr. Charlene Rapsey was responsible for guiding the selection of cognitive tests, test design, testing procedures and the methods of scoring. This study was part of a larger trial to assess an array of associations with the Glycaemic Index. The candidate and Fiona Kendall (another MDiet candidate) worked together on preparatory procedures for this study that involved screening and recruiting participants and the development of the test food. The candidate's individual responsibilities involved the cognitive function component of this study.

The candidate was responsible for:

- Examining and writing a review on the literature on the glycaemic index and cognitive function
- Input into study design, preparatory procedures; and cognitive test selection and procedures
- Preparing and designing cognitive tests
- Participant recruitment and instruction including communicating study procedures in lectures; and ongoing online, written, and verbal communication throughout the trial period
- Organising and providing breakfasts and scales to participants

- Trifle recipe design including ingredient and nutrient calculations; ingredient and equipment sourcing and purchasing; baking for test food planning, GI testing and study testing sessions; and accommodating for special dietary needs
- Liaising with staff involved in testing day facilitation, blood collection, IT systems and blood analysis
- Assembling and designing cognitive tests into timed slides to ensure standardisation of test times and smooth running of all sessions and printing test papers
- Setting up and conducting testing sessions, implementing and instructing the cognitive tests and collecting blood
- Data collection, collation and entry of the data
- Input into statistical analysis with biostatistician
- Interpreting the results of the study
- Writing this thesis

Acknowledgements

I would like to take the opportunity to express my gratitude to all who have helped me to complete this thesis.

To Dr. Bernard Venn, your wealth of knowledge and expertise have helped guide me to the completion of this thesis. Thank you for taking the time to give support and feedback throughout this process.

To Dr. Charlene Rapsey, your guidance on cognitive testing and your feedback was invaluable. Thank you for all of your help.

To Dr. Jill Haszard, thank you for your work on the statistical analysis. I am very grateful that you took the time to explain the processes.

To the laboratory team, Kieran, Ivy, Ken, and Margi, thank you for all that you did to ensure the laboratory preparations and laboratory sessions ran smoothly. A lot of time and effort was involved in this and that did not go unnoticed.

To Ashley Duncan, thank you for processing the bloods and taking the time to show and explain the processes to us.

To Frances Mosley, thank you for preparing and processing all of our purchase orders.

To fellow MDiet candidate, Fiona Kendall, this research process was especially enjoyable working with you. Thank you for all of your help and support throughout!

Finally, thank you to my partner Jack and our son Hector, your support throughout this thesis process and all of my studies has made it possible to get to this point. You allowed me the time to work hard and you always ensured I had enjoyable breaks in your company!

Table of Contents

Abstract	ii
Preface	iv
Acknowledgements	vi
Table of Contents	vii
List of Tables	x
List of Figures	xi
List of Abbreviations	xii
1. Introduction	1
2. Literature Review	3
2.1 Introduction	3
2.2 Literature Review Methodology	3
2.3 Glycaemic Response	3
2.3.1 Glucose.....	3
2.3.2 Glycaemic Response	4
2.3.3 Glycaemic Index	5
2.3.4 Glycaemic Load	6
2.4 Sucrose and Isomaltulose (Palatinose®)	6
2.5 Brain Energy Metabolism	8
2.6 Glycaemia and Cognitive Function	9
2.6.1 Suggested Associations between Postprandial Glycaemia and Cognitive Function ...	9
2.6.2 Previous research on glycaemia and cognitive function	10

2.6.3 Rationale for this research.....	20
3. Objective Statement.....	21
4. Subjects and Methods.....	22
4.1 Study Design	22
4.2 Test Food	22
4.3 Cognitive Tests.....	24
4.4 Research Procedures	29
4.4.1 Randomisation.....	29
4.4.2 Double Blinding.....	29
4.4.3 Data Collection.....	29
4.4.4 Preparatory Procedures	29
4.4.5 Demographics Collection.....	30
4.4.6 Testing Day Procedure.....	30
4.4.7 Blood Collection and Analysis.....	31
4.5 Statistical Analysis.....	32
4.5.1 Sample Size Estimation.....	32
4.5.2 Statistical Analysis	32
5. Results	33
6. Discussion	37
6.1 Recommendations.....	41
6.2 Conclusion	43
7. Application to Dietetic Practice	44
7. References	45
8. Appendices.....	51
Appendix A: Ethics Proposal and Approval Letter	52

Appendix B: Trifle Recipe and Nutrient Information	54
Appendix C: Wordlists for Free Word, Short Delay and Long Delay Recall Tests.....	55
Appendix D: Visuospatial Test Example.....	56
Appendix E: Participant Demographics Form	59
Appendix F: Consent Form	60
Appendix G: Westgard Rules.....	62
Appendix H: Roche Cobas C Glucose Analysis Procedure	66

List of Tables

Table 2.1 Associations Identified in Research between GI and GL and Better Speed of Processing	13
Table 2.2 Associations Identified in Research between GI and GL and Better Working Memory Performance	15
Table 2.3 Associations Identified in Research between GI and GL and Better Inhibitory Control	16
Table 2.4 Associations Identified in Research between GI and GL and Better Immediate Word Recall	18
Table 4.1 Cognitive Tests.....	24
Table 5.1 Demographic Characteristics of Participants.....	34
Table 5.2 Adjusted mean differences in cognitive test scores between sugars at 60 and 120 minutes*	36

List of Figures

Figure 2.1 Chemical Structure of Sucrose and Palatinose	7
Figure 2.2 Glycaemic Response to 50 g Sucrose and Isomaltulose.....	8
Figure 4.1 Glycaemic Response Curve to Sucrose and Isomaltulose Trifles with 50 g of Available Carbohydrate	23
Figure 4.2 Test Session Timeline.....	25
Figure 5.1 Study Design and Participant Flow Diagram	33
Figure 5.2 Blood glucose concentrations at each cognitive test period	35

List of Abbreviations

AUC	Area under the curve
GI	Glycaemic Index
g	Grams
m	Metres
RCF	Relative Centrifugal Force
RCT	Randomised Controlled Trial
s	Seconds

1. Introduction

The glycaemic index (GI) is a concept that has been studied by numerous researchers to identify any health benefits or detriments that can be attributed to it (Goff, Cowland, Hooper, & Frost, 2013; Livesey, Taylor, Hulshof, & Howlett, 2008; Mulholland, Murray, Cardwell, & Cantwell, 2008; Thomas, Elliott, & Baur, 2007). A trending theory is that low GI foods supply longer lasting energy that consequently maintains cognitive function over time as opposed to a more rapid decline in energy and cognitive performance after high GI foods (Glycemic Index Foundation, 2017). Benefits to some areas of cognitive function are reported from research articles, some of which are cited by the Glycaemic Index Foundation to support their health message that a low GI diet can improve cognitive function (Glycemic Index Foundation, 2017). However, the consistency of findings is questionable due to variable study designs and contradicting results. Thus, existing research has highlighted a possible relationship between low GI foods and cognitive function but cause and effect has not been confirmed.

The theory on GI and cognitive function has developed from a combination of previous findings. Firstly, the brain solely relies on the metabolism of glucose for energy in its non-prolonged fasting state (van de Ven, van der Graaf, Tack, Heerschap, & de Galan, 2012). This, in conjunction with findings that breakfast consumption as opposed to fasting and skipping breakfast may reduce cognitive decline throughout the morning, indicates the possibility that circulating glucose levels may contribute to this effect (Liu, Hwang, Dickerman, & Compher, 2013; Pollitt, 1995; Pollitt, Lewis, Garza, & Shulman, 1982).

To further legitimise this theory, a symptom of hypoglycaemia in people with Type 1 diabetes is cognitive decline (Inkster & Frier, 2012). This symptom has also

been detected in healthy people in an induced hypoglycaemic state (Graveling, Deary, & Frier, 2013). However, non-diabetics do not typically experience hypoglycaemia due to the homeostatic systems in place to prevent it (Cryer, 2017). Therefore, the question remains whether cognitive function is affected at varying euglycaemic levels induced by foods differing in GI.

The main issue with the body of research on this topic is much of the evidence has emanated from studies that lack control of nutrient content in the test meals, for example fibre or protein content, so any results cannot conclusively be attributed to varying GI.

Additionally, it is undetermined to what extent the GI theory may apply. Cognition in relation to GI has primarily been examined in children and adolescents who may respond differently to adults (Philippou & Constantinou, 2014). The aim of this study is to determine whether GI independently influences cognitive performance in an adult population using a study design that controls for all variables other than a difference in glycaemic response.

2. Literature Review

2.1 Introduction

The focus of this literature review is on the effect that postprandial glycaemia has on cognitive function.

The aim of this literature review is to:

1. Provide an overview of the glycaemic response, the glycaemic index, sucrose and isomaltulose biochemistry and metabolism, and the brains energy metabolism.
2. Outline the association between the glycaemic response and cognitive functioning.
3. Discuss the current research on glycaemia and cognitive function.
4. Determine the need for research assessing postprandial glycaemia and cognitive function.

2.2 Literature Review Methodology

For the collection of appropriate studies for review, the databases Medline via Ovid, Scopus, and PubMed were searched using the keywords: 'blood glucose', 'dietary carbohydrates', 'glyc(a)emic index', 'glyc(a)emic load', 'isomaltulose', 'palatinose', 'cognition', 'memory', 'cognitive function'. Appropriate literature referenced in the collected articles was also collected. Only research written in English and based on humans were included.

2.3 Glycaemic Response

2.3.1 Glucose

Glucose is a simple sugar, monosaccharide carbohydrate present in many foods. Glucose can be consumed in its monosaccharide form as free glucose, but more

commonly it is consumed as sugar disaccharides, or starch oligosaccharide or polysaccharide (Cummings, 2012). These are chains of monosaccharide carbohydrates including glucose, fructose and galactose bonded together by glycosidic linkages (Cummings, 2012).

Carbohydrates provide approximately 46 % of New Zealanders calorie intake (University of Otago and Ministry of Health, 2011). Many foods contain glucose therefore postprandial elevation in blood glucose is normal (Jenkins, 1981).

Ingestion of glucose-containing foods results in glucose absorption that will cause a temporary rise in blood glucose concentrations once the glucose is absorbed from the intestinal tract into the blood stream (Holmes, 1971). In order for monosaccharides to be absorbed into the blood stream, the glycosidic linkages must be hydrolysed by specific enzymes to release them from the chain (Goñi, Garcia-Alonso, & Saura-Calixto, 1997).

2.3.2 Glycaemic Response

The extent and rate of the rise and fall of blood glucose concentrations after absorption of a food or meal is referred to as the glycaemic response (L. S. A. Augustin et al., 2015; Blaak et al., 2012). The glycaemic response to different foods vary. The rise in blood glucose levels is determined by the quantity of glucose present in a food and the physical properties that determine transit time through the gastrointestinal tract (L. S. Augustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002). Longer transit time results in slower rates of food hydrolysis with the eventual release of individual glucose monomers for absorption (L. S. Augustin et al., 2002). Such physical properties that affect glycaemic response include meal size; rheological properties; level of disruption to botanical structure; level of starch hydration; presence of lipid, protein, acid and

fibre; carbohydrate chain length, and bond type; and presence of inhibitors to enzyme action (Bjorck, 1994; Jenkins et al., 1982; Welch, Bruce, Hill, & Read, 1987; Zhu, Hsu, & Hollis, 2013).

Nutrients other than glucose cannot raise blood glucose levels directly. However, some amino acids, lipid fractions and fructose can be transformed and converted into glucose through the process of gluconeogenesis which occurs in the liver and kidneys and results in the release of glucose into the bloodstream to prevent hypoglycaemia (Sun & Empie, 2012). This effect is not included in the glycaemic response as it is not principally a postprandial response (L. S. A. Augustin et al., 2015).

Rapid rises in blood glucose concentrations can lead to a responsive spike in insulin levels to stimulate uptake of glucose into the cells to prevent hyperglycaemia (Gagne, 2008). However, high insulin concentrations can cause blood glucose concentrations to temporarily drop below baseline (Gagne, 2008). In contrast, slower and more steady rises in blood glucose concentrations cause a less pronounced insulin spike and slower peripheral uptake of glucose from the blood with little or no drop in glucose levels below baseline (Du, Van Der A, & Feskens, 2006; Ludwig, 2002).

2.3.3 Glycaemic Index

The glycaemic index (GI) is a comparative classification of the glycaemic response to a fixed amount of available carbohydrate in a food relative to a standard reference food containing the same amount of carbohydrate (L. S. A. Augustin et al., 2015). It quantifies the relative glycaemic response following consumption of a test food to that of the reference food containing the same amount of carbohydrate over a two hour period (Jenkins, 1981).

GI is the incremental area under the glycaemic response curve (AUC) after ingestion of 50 g of available carbohydrate from a food in a human subject, divided by the AUC after 50 g of carbohydrate from a reference substance (either pure glucose or white bread) in the same person (Wolever, 1991). The final GI value is the average of at least ten healthy volunteer's glycaemic response to the test food in comparison to the control substance. It is expressed as a percentage of the reference food response (Wolever, 1991).

A food is classified as high GI if the value is 70 or greater, medium GI if 56-69 and low GI if 55 or less (Brand-Miller, 2003).

2.3.4 Glycaemic Load

GI can be further classified into glycaemic load (GL). GL is an extension of GI which includes the amount of available carbohydrate consumed (Salmerón et al., 1997; Wolever, 1991). This is often used because the proportions of available carbohydrates differ between different foods, and thus reflects the glycaemic response to an average, or given, serving size of a food or meal (Gagne, 2008).

2.4 Sucrose and Isomaltulose (Palatinose®)

Sucrose is a carbohydrate that is present naturally in fruit and vegetables and is extracted from sugar cane and sugar beet to make table sugar (PubChem Compound Database). Sucrose is a disaccharide composed of one glucose and one fructose molecule bonded by an α -1,2-glycosidic bond (PubChem Compound Database).

Isomaltulose is also a disaccharide sugar composed of glucose and fructose but differs to sucrose by its more slowly digested α -1,6-glycosidic bond (PubChem Compound Database). Isomaltulose is present naturally in honey and sugar cane in small amounts and it is produced as a commercial alternative sugar, marketed as

Palatinose[®] (Lina, Jonker, & Kozianowski, 2002). Palatinose is formed by enzymatic rearrangement of the glycosidic bond in sucrose, followed by crystallisation (Lina et al., 2002; Maeda et al., 2013) (**Figure 2.1**).

The glycosidic bond in isomaltulose is more slowly hydrolysed into its monosaccharide components in the human small intestine than sucrose (Dahlqvist, 1963). The difference is attributed to the sugars responding to different brush border enzymes that function at different rates to catalyse the hydrolysis of their bonds. Bond hydrolysis in sucrose is catalysed by sucrase (invertase) more rapidly than isomaltulose is by isomaltase (Dahlqvist, 1963). Slower bond hydrolysis of isomaltulose generates a more prolonged delivery of glucose to the bloodstream thus a low GI that is half that of sucrose's despite their glucose content being the same (GI=32 and 65, respectively) (Brand-Miller J, 2016; Lina et al., 2002).

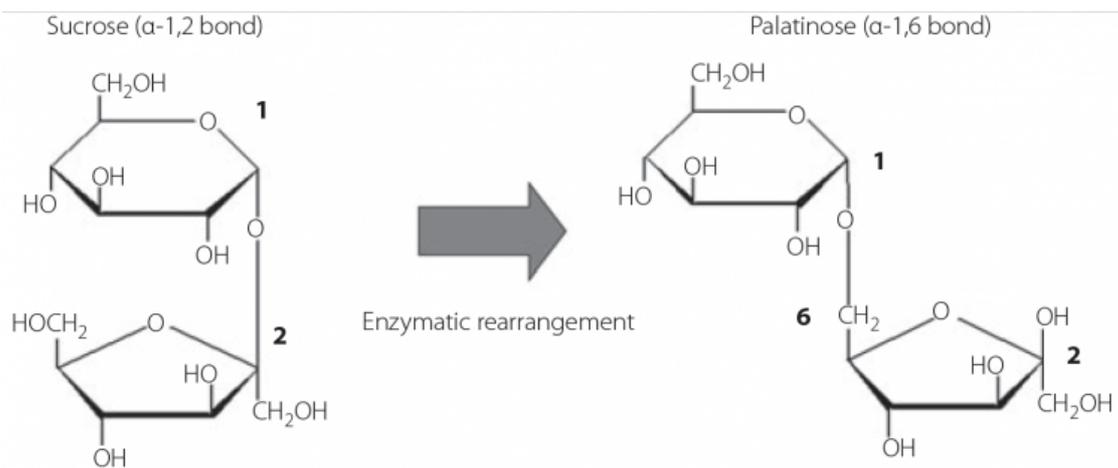


Figure 2.1 Chemical Structure of Sucrose and Palatinose (Maeda et al., 2013)

It has been said that the glycaemic response to high doses of sucrose includes levels that eventually fall below baseline whereas with isomaltulose it does not, as displayed in **Figure 2.2** (Holub et al., 2010; Ludwig, 2002; Maeda et al., 2013).

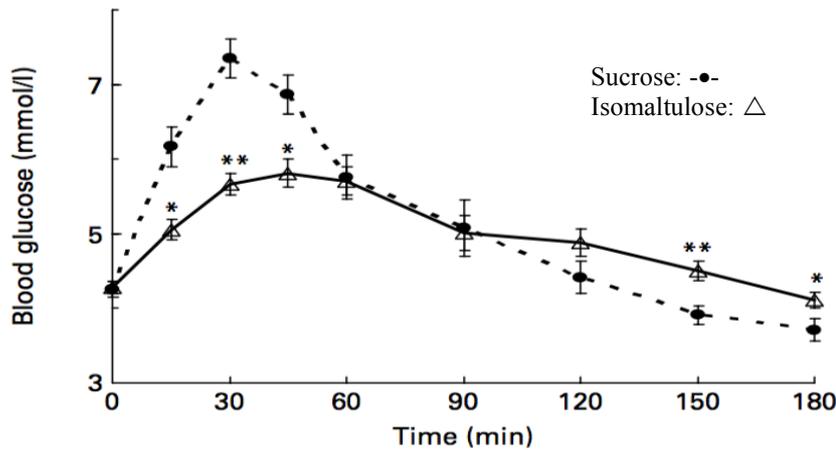


Figure 2.2 Glycaemic Response to 50 g Sucrose and Isomaltulose
 Mean values were significantly different: *P<0.05, **P<0.01 by Wilcoxon test for paired data.
 (Holub, 2010)

2.5 Brain Energy Metabolism

In a normal, non-prolonged fasting state almost all oxidative metabolism in the brain can be attributed to the metabolism of glucose (Lattera, Betz, Lorriss, & Goldstein, 1999). The energy requirement of the brain is 20 % of whole body energy consumption which is disproportionately large considering its weight is 2 % of body weight (Lund-Andersen, 1979). Little glucose can be stored in the brain (Lund-Andersen, 1979). At basal metabolic rate, stores of glucose in the brain are estimated to be exhausted in ten to fifteen minutes (Lund-Andersen, 1979). Thus, the brain relies on a continuous supply of glucose from the blood (Sünram-Lea, 2015).

Glucose enters the brain by facilitated diffusion using GLUT 1 receptors in the blood-brain barrier leading to equilibrium between blood and brain glucose concentration rather than glucose accumulation (Hasselbalch, 1994; Simpson, Carruthers, & Vannucci, 2007). Studies in humans have shown a linear relationship between brain glucose concentrations and plasma glucose concentrations ranging from 4.6 to 30 mmol/L, measured using magnetic resonance spectroscopy (Choi, Lee, Kim, & Gruetter, 2001; De Graaf et al., 2001; Gruetter, Ugurbil, & Seaquist, 1998). There is

uncertainty over whether this relationship exists at hypoglycaemic levels as measurement in this state is challenging. This is because infusion of glucose substrate is required which conflicts with obtaining hypoglycaemia (van de Ven et al., 2012). Data from one study are suggestive of a linear relationship at hypoglycaemic levels to 3mmol/L, although the authors needed to make a number of assumptions in arriving at this conclusion (van de Ven et al., 2012).

2.6 Glycaemia and Cognitive Function

2.6.1 Suggested Associations between Postprandial Glycaemia and Cognitive Function

It has been proposed that cognitive function is affected by blood glucose concentrations because the brain is reliant on glucose to function in the non-fasting state (Lattera et al., 1999). It is also claimed that the rate of glucose metabolism is accelerated in task specific areas of the brain which suggests that fluctuations in the availability of glucose may affect brain metabolism and consequently cognitive function (Lund-Andersen, 1979). Furthermore, studies have shown positive effects on cognition with glucose loading in comparison to meal omission or a placebo (Scholey, Harper, & Kennedy, 2001). All factors indicate that the glycaemic response to foods could play a role in cognition.

The scientific body of literature has been cited by a not-for-profit organisation, the Glycaemic Index Foundation, with claims that low GI foods improve cognitive performance (Glycemic Index Foundation, 2017). Despite the suggestions, conclusive evidence to support such claims are lacking with an unclear relationship between the GI of a meal and its effects on cognitive function. However, the following associations

between GI and cognitive functioning could be proposed based on the relationship between blood glucose concentrations and the energy metabolism of the brain:

- There is no association between the GI of a food and cognitive function because blood glucose levels do not fall low enough for functioning to decline.
- Low GI foods facilitate better cognitive functioning postprandially than higher GI foods because low GI foods do not cause blood-glucose concentrations to fall below baseline levels as do high GI foods, prolonging supply of glucose to the brain thus improving cognitive function.
- Initially, high GI foods promote better cognitive functioning postprandially than low GI foods because high GI foods produce rapid and high blood-glucose concentrations, supplying more glucose to the brain thus improving cognitive function.

2.6.2 Previous research on glycaemia and cognitive function

The relationship between GI and cognitive function has been assessed using numerous cognitive tests. Tests can be summarised to represent a cognitive process. Overall, the evidence for any associations are weak due to difficulties in study design and conflicting outcomes.

All of the research cited by the Glycaemic Index Foundation to support low GI claims on this topic are studies in children and young adolescents and the effects of breakfasts varying in GI and/or GL on cognitive function (Glycemic Index Foundation, 2017). Children are not a representative sample of the human population as the tasks tested may be more difficult due to their young age and the more difficult a task the more glucose may be required (Draelos et al., 1995; R. Manning, 1982; Scholey et al.,

2001). Furthermore, as only breakfasts were assessed, it cannot be assumed that any effects of GI occur after all meals throughout the day.

A randomised control trial (RCT) that examines the effects of high and low GI and GL breakfasts in young adolescents is cited by the Glycaemic Foundation to support the claim that low GI diets improve cognitive performance (Micha, Rogers, & Nelson, 2011). This study had one of the better study designs of all the four articles cited by the Foundation as it was an RCT. The findings were summarised by the Foundation as “low GI meals predicted better declarative-verbal memory with the overall conclusion being that the low GI, high GL breakfasts may help to improve learning.” (Glycemic Index Foundation, 2017). However, from the variety of tests undertaken, low GI was only associated with a better performance of a word generation task which assess cognitive flexibility ($P=0.03$) (Diamond, 2013). On the other hand, high GI meals predicted better performance on a Stroop test (high GL meals only), speed of processing and serial sevens tasks (both regardless of GL). Thus, participants performed better on tests assessing three areas of cognitive function (inhibitory control as assessed by a Stroop test, working memory as assessed by the serial sevens task, and speed of processing) after the high GI meals whereas after the low GI meal performance was better on one area of cognitive functioning (cognitive flexibility as assessed by a word generation task) (Diamond, 2013).

However, neither finding can definitively be attributed to GI as the low and high GI breakfasts did not cause significant differences in blood glucose concentrations at the time of cognitive testing. Furthermore, the results are confounded by differing energy and nutrient content of the meals.

Other studies, including the remaining studies cited by the Glycaemic Index Foundation, have found varying and contradicting associations between GI and cognitive test data and discussions of these follow.

Speed of Processing

A parallel (between-subject design) study in 11-14 year old adolescents found low GI and high GL breakfasts were associated with better speed of processing ($P=0.031$ and $P=0.001$, respectively) (Micha, Rogers, & Nelson, 2010). In this study, researchers recorded what the participants had for breakfast and classified them into low and high GI and GL groups. The correlations were highly subject to confounders because between-subject comparisons were made and all breakfasts within and between GI and GL groups varied in food type, size, and energy and nutrient content. The correlations were further weakened because the participants sat the cognitive tests at different times and due to the nature of the glycaemic response curve, testing at different times could cause varying results if blood glucose concentrations predict performance.

In another study with a balanced cross-over design, speed of processing in 75 children aged 5-11 years old was independent of the GL of the meals (Young & Benton, 2014a). In secondary analysis, the authors reported that speed of processing was faster following a low compared with a high GL breakfast in participants who received the low GL breakfast on their second testing day ($P<0.0001$). The meaning of this finding is unclear because the association was dependent on order of treatment.

Blood glucose concentrations were not measured either, thus, it cannot be confirmed the different GL caused differing glycaemic responses to have a potential effect on scores.

Table 2.1 demonstrates the significant associations detected in the studies discussed and displays the variability in the findings. The parallel study by Micha et al. (2010) has a weaker study design to the crossover studies. However, as all studies contained substantial confounders and differences in blood glucose concentrations were either not measured or detected, none of the findings are robust.

Table 2.1 Associations Identified in Research between GI and GL and Better Speed of Processing

Reference	Factor associated with superior performance				No Association
	Low GI	High GI	Low GL	High GL	
(Micha et al., 2010)	✓			✓	
(Micha et al., 2011)		✓			
(Young & Benton, 2014a)	-*	-	✓		

*Dashes indicate that the variable was not assessed in the study

Working Memory

In a parallel study of adolescents, low GI breakfasts were also associated with better performance in the serial sevens test, a working memory task (Micha et al., 2010). This finding is opposite to that found by the same authors in 2011, in which the serial seven test was better performed following the high GI treatment (Micha et al., 2011). The results from a randomised cross-over trial in healthy adult males support this finding to some extent (Dye, 2010). A sucrose-sweetened milk produced the highest glycaemic response and was associated with better performance of the serial sevens task 35 minutes following consumption compared to an isomaltulose-sweetened milk and a water control. The isomaltulose-sweetened milk produced a higher glycaemic response after 35 minutes than the water but no difference in performance between the two milk drinks was detected. These findings could suggest blood glucose concentrations higher than what 50 g of isomaltulose caused are required to improve working memory performance. However, in a secondary analysis the significant difference was only

identified in those with lower scores in their baseline test ($P=0.0034$). The study was not powered to assess this, thus further investigation of this relationship is required to make any conclusions.

There are also clear learning effects with the serial sevens test as it involves successive subtraction of seven beginning at 100 so the participant would rely on memory of their previous test attempts (R. Manning, 1982). This effect was observed in this study as a statistically significant trend for increasing scores over each consecutive visit was detected ($P=0.025$), thus cognitive scores of the participants depended on treatment order.

Baseline tests were performed, although it was not established whether the cognitive ability of the participants differed between order. Thus, a group assigned to receive treatment in one order may gain learning effects from the test at a different pace than another.

In another study, it was claimed that calculation ability, another working memory task, declined less over time after drinking a low GI water sweetened with isomaltulose as opposed to drinking a higher GI sucrose-sweetened water beverage in a small parallel study (Kashimura, Nagai, & Ebashi, 2003). The researchers noted that scores decreased less on the third test in the low GI group than the high GI, but no statistical significance was found. As there were only fourteen participants in the study and the comparisons were between participants the differences were likely due to the individual calculation ability rather than their glycaemic response.

A test beverage like that in the studies by Dye et al. (2010) and Kashimura et al. (2003) is ideal to determine whether the effects are due to GI and is needed to improve the quality of both studies by Micha et al. (2010, 2011). However, the study by Dye et al. (2010) was limited by post-hoc findings and the influence of learning effects.

Comparison of baseline scores is needed to determine if cognitive ability is a confounding variable. Thus, the finding by Dye et al. (2010) should be viewed as inconclusive. **Table 2.2** demonstrates variability of the findings discussed.

Table 2.2 Associations Identified in Research between GI and GL and Better Working Memory Performance

Reference	Factor associated with superior performance				No Association
	Low GI	High GI	Low GL	High GL	
(Micha et al., 2010)	✓				
(Micha et al., 2011)		✓			
(Dye et al., 2010)		✓	-*	-	
(Kashimura et al., 2003)			-	-	✓

*Dashes indicate that the variable was not assessed in the study

Inhibitory Control

Inhibitory control has been assessed by the Stroop test in three studies and each reported different findings. There was no association between GI or GL, and response time and accuracy on the Stroop test in the parallel study in adolescents (Micha et al., 2010). In an RCT in adolescents, better performance was associated with a high GI breakfast (Micha et al., 2011). However, in a more recent study low GI breakfasts have been associated with better performance in the Stroop test (Cooper, Bandelow, Nute, Morris, & Nevill, 2015). In this study, 42 young adolescents were assigned to receive a low or high GI breakfast. The breakfasts comprised different foods but all were similar in energy and macronutrient content. The participants received the same breakfast on two separate occasions. On one occasion, they rested and on the other they exercised between their first and second cognitive test battery. A significant breakfast by exercise by session time interaction was found on the complex level of the Stroop test (P=0.012). The researchers concluded that a low GI breakfast coupled with a morning bout of exercise benefited performance on the Stroop test. However, the differences in order

from greatest to least benefit on performance was low GI with exercise, followed by high GI with rest, then low GI with rest, then high GI and exercise. A trend for benefit of GI level, or the effects of level of activity in relation to meal GI, is not evident from this order which makes it difficult to draw conclusions from the observed associations.

Table 2.3 demonstrates the conflicting findings discussed. The influence of GI in performance on the Stroop task is unclear as findings are inconsistent across the three extant studies.

Table 2.3 Associations Identified in Research between GI and GL and Better Inhibitory Control

Reference	Factor associated with superior performance				No Association
	Low GI	High GI	Low GL	High GL	
(Micha et al., 2010)					✓
(Micha et al., 2011)		✓			
(Cooper et al., 2015)	✓ ^a		-*	-	

^aAssociation identified on the complex level of Stroop test only.

*Dashes indicate that the variable was not assessed in the study

Cognitive Flexibility

In the RCT study by Micha et al. (2011), a low GI breakfast predicted better performance on the word generation task in an adult sample. This finding was not replicated in a parallel study with adolescents; in this study no association between GI and performance on the word generation task was detected (Micha et al., 2010). Controlling the nutrient and size content of the meals and instigating a difference in glycaemic response after the two meals is needed to determine if either finding is reliable.

Immediate and Delayed Word Recall

Three studies were identified that examined performance on an immediate word recall task; no clear pattern of association between performance and GI was observed across these studies. High GI breakfasts were associated with better performance in children in

a parallel study in adolescents (Micha et al., 2010). No association was found in an RCT in adolescents (Micha et al., 2011), whereas in another parallel study, a low GL breakfast was associated with better recall in middle and older age adults who simultaneously had better glucose tolerance and their lowest blood glucose level remained above baseline during a 2-hour glucose tolerance test (Young & Benton, 2014b). In this study, participants were served a breakfast that only differed by the sugar it was sweetened with. The low GL breakfast was sweetened with isomaltulose, the medium GL with sucrose and the high GL with glucose. The study found a positive association between score and the low GL breakfast compared to the medium at all immediate word recall tests (30, 115 and 195 minutes post breakfast) and at the last two time points between the low and high GL breakfast in participants with better glucose tolerance that remained above baseline.

These findings are in different age groups so it could indicate that GI affects cognition differently in different age groups or glucose tolerance could play a role in cognitive function. But, both studies are parallel, so cause and effect cannot be inferred from either. The middle and older age adult study had several strengths including, the breakfasts were matched for physical size, energy and nutrient content, unlike the adolescent study. However, the highest GL meal contained different amounts of glucose and fructose than the medium and low GL meal which confounds the high GL finding. Furthermore, blood glucose was not measured after the breakfast so it cannot be confirmed that the breakfasts caused different glycaemic responses. **Table 2.4** demonstrates the variability of the findings.

None of these studies found any association between GL and delayed word recall but a better study design would allow determination of the reliability of this finding.

Table 2.4 Associations Identified in Research between GI and GL and Better Immediate Word Recall

Reference	Factor associated with superior performance				No Association
	Low GI	High GI	Low GL	High GL	
(Micha et al., 2010)					✓
(Micha et al., 2011)		✓			
(Young & Benton, 2014b)	-*	-	✓		

*Dashes indicate that the variable was not assessed in the study

Immediate and Delayed Visuo-spatial Memory

In a study with 19 children aged 5-7 years old, a lower GL breakfast was associated with better immediate visuo-spatial memory scores ($P < 0.04$) (Benton, Maconie, & Williams, 2007). In Benton's study, participants were supplied with two of three different breakfasts on separate occasions that were similar in energy content and were either low, medium, or high GL and subsequently performed the visual memory test sometime after each. This indicates that only 12-13 participant scores for each GL category were available for comparison. Also, the comparisons were not all within-subject. Because between-subject comparisons were made and the sample size was small, potential for confounding is high. Specifically, the cognitive ability of the participants would confound the results.

Furthermore, the time of testing was not set, thus differences in performance could be due to the time of the test rather than the GL of the meal. Additionally, the physical size and the macro and micro nutrient content of the meals differed which means difference in performance cannot only be attributed to GL.

The relationship between visuo-spatial memory was investigated further in a RCT in children aged 5-11 years old, which was of higher quality, and a similar association was found (Young & Benton, 2014a). There was no difference between the high and low GL breakfasts and visual memory one hour after breakfast intake.

However, after three hours, visual memory was better maintained on the low compared to the high GL breakfast ($P < 0.001$). This suggests that the low GL breakfast may have supplied more glucose at this point which attributed to better performance. However, conclusions regarding the influence of GI are limited as blood glucose concentrations were not measured and the breakfasts differed in amount of glucose and fructose and in physical size.

Neither of these studies found an association between GL and delayed visuospatial memory.

The findings from both studies favour low GL breakfasts for immediate visuospatial recall and neither provide evidence of an effect of GL on delayed recall. Though, the findings are not completely convincing due to confounding. The finding at three hours post breakfast consumption is the most promising thus far but controlling the food so that rate of glucose absorption is the only difference would produce a more rigorous result (Young & Benton, 2014a).

Combined Cognitive Process Tests

Reitan's trail test B is a test of visual attention, speed of processing and cognitive flexibility (Broshek & Barth, 2000; Salthouse, 2011). It has been associated with poorer performance during hypoglycaemia (2.2 mmol/L) compared to euglycaemic levels in type 1 diabetics (Draeos et al., 1995). The decrement in performance was greater than that of other tests of cognition, including some of the test discussed above. It was suggested that the increased level in difficulty of this test explains the decline in performance. This corresponds with the theory that glucose facilitates cognitive function on demanding tasks (Smith, Riby, Eekelen, & Foster, 2011). However, interstitial glucose was measured at three minute intervals in a GI and cognition study (Dye et al., 2010). Reductions in circulating glucose were not detected during the

demanding cognitive tests. One possible explanation is that the completion of such tasks does not increase brain glucose requirements. An alternative explanation is that the difficulty level of Reitan's trail test B is greater, thus increasing glucose demand.

It has also been suggested that tasks of divided attention are facilitated by glucose, although the evidence is stronger in older adults and patients with Alzheimer's disease (C. A. Manning, Parsons, Cotter, & Gold, 1997; C. A. Manning, Ragozzino, & Gold, 1993). None of the research discussed has assessed performance on a task under condition of divided attention thus further study on divided attention in the healthy younger population is of interest.

2.6.3 Rationale for this research

The contradictory results produced by past studies on the relationship between GI/GL and cognitive function explain the need for further research to determine whether the GI/GL of a food affects cognitive performance. The study designs and potential for confounding are likely reasons for variable outcomes. Thus, in order to identify convincing associations, a study is needed that uses a meal that differs only in the rate of absorption between the high and low GI meal. It is possible to design an appropriately powered RCT in which the comparison foods are identical in all aspects except the glycaemic properties.

3. Objective Statement

The aim of this study is to test whether foods differing in glycaemic index influence cognitive performance.

The objectives of this study are to:

- To develop an iso-energetic, identical nutrient, palatable food containing either the sugar sucrose or isomaltulose, that differs only in the rate of digestion of the two sugars.
- To determine whether memory and speed of processing differ in response to consuming the two test foods.

4. Subjects and Methods

This study was undertaken in the Human Nutrition Undergraduate Laboratories at the University of Otago from 03rd-31st of March, 2017.

Approval for this study was granted by The University of Otago Human Ethics Committee (Appendix A).

4.1 Study Design

This study is a double-blinded, randomised, controlled, crossover trial of cognitive function with repeated measures over time in relation to postprandial glycaemia after a sucrose- or isomaltulose-sweetened trifle.

Students from a 300-level undergraduate human nutrition course at the University of Otago, New Zealand, were invited to participate in this study during their course laboratory sessions. The exclusion criteria included anyone who had been diagnosed with diabetes, and anyone who was colour-blind would be excluded from one cognitive test that required colour identification.

Participants ate a sucrose-sweetened trifle at one testing session and a isomaltulose-sweetened trifle at the other. A battery of cognitive tests was completed by participants at baseline (before food consumption) and at one and two hours after consumption.

Another candidate implemented their study alongside this study using the same participants and test food to assess the effects of glycaemia on satiety.

4.2 Test Food

The test food used in this study was trifle sweetened with either 98.8g of sucrose or isomaltulose. Trifle was chosen as it was considered: filling (for the purpose of the satiety study); easily alterable to suit special dietary needs; and it optimised the amount of sugars contained with a high pure sugar to total carbohydrate, fat and protein.

The trifles differed only by the type of sugar used. The ingredients, recipe and nutrient content are included as Appendix B. Nutrient content was calculated from the New Zealand Food Composition Tables 2016 (S. Sivakumaran & L Huffman, 2017).

Smaller trifles were made in serving sizes that contained 50g available carbohydrate for GI testing, in accordance with standard procedure. The carbohydrate content was tested and confirmed by Dr. John Munro from Plant and Food Research, NZ. It was also confirmed that the two trifles did not differ in nutrient content.

Glycaemic Index Otago, NZ determined the GI from the glycaemic response in twelve subjects. The sucrose trifle had a GI of 44 and the isomaltulose trifle had a GI of 33. The postprandial glycaemic response to each trifle over the two-hour period is displayed in **Figure 4.1**.

Participants who had special dietary needs were served either vegan jelly or custard and jelly which contained the same amount of sugar as the trifles.

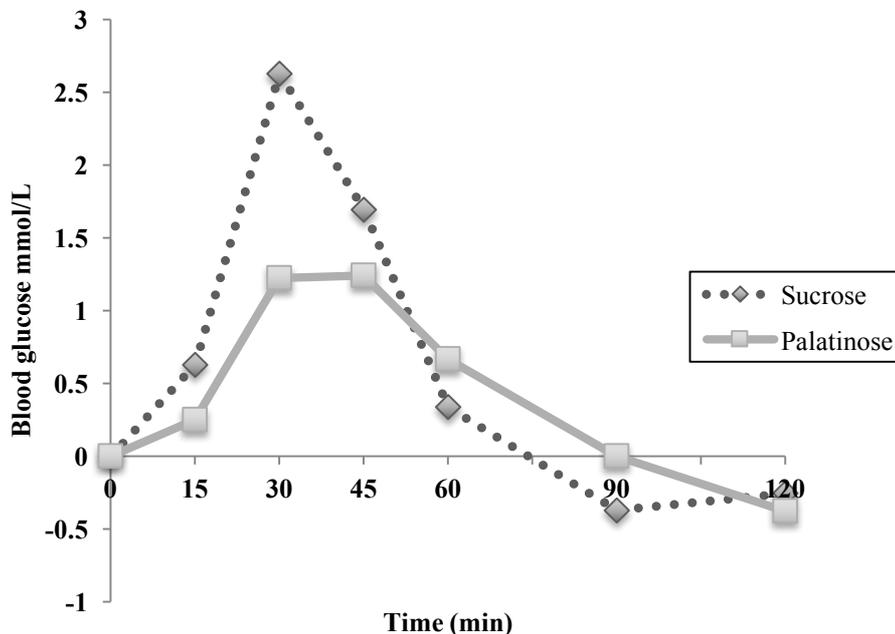


Figure 4.1 Glycaemic Response Curve to Sucrose and Isomaltulose Trifles with 50 g of Available Carbohydrate

4.3 Cognitive Tests

Six different cognitive tests were performed in the laboratories as described in **Table 4.1**. Each participant filled answers to the cognitive tests on test papers which were collected immediately after each individual test. Two projector screens in clear view of all participants were used to display test content. The laboratory was fitted with surround speakers and were used to ensure all test content could be heard.

The content used in the tests on each testing day was different to ensure that no participant would be at an advantage if they heard about the tests from other participants before their own testing day.

Table 4.1 Cognitive Tests

Test	Outcome	Max. score
Free Word Recall	Number of words recalled under a test condition of divided attention	20
Short Delay Recall	Number of words recalled from free WR* after a time lapse of 5 minutes	20
Long Delay Recall	Number of words recalled from free WR after a time lapse of 20 minutes	20
Letter-Number Sequence Recall	Number of correct sequences consecutively recalled	8
Visuospatial Recall	Number of correct observations	5
Reitan's Trail Making Test Part B	Time to complete	N/A

*WR, Word Recall

Timing of Cognitive Tests

Figure 4.2 outlines the order of events at the test sessions. Five of the tests: free word recall, short delay word recall, long delay word recall, letter-number sequence and visuospatial recall visuospatial were performed at baseline, and 60 and 120 minutes after the participants began eating the trifle. Reitan's trail making test part B was performed as the last test of the 60-minute cognitive test battery.

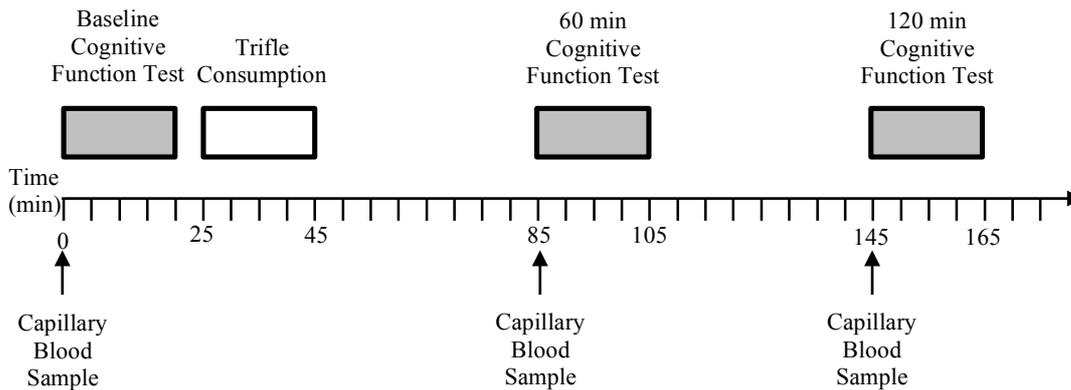


Figure 4.2 Test Session Timeline

Word Recall Tests

Word recall tests assess immediate and delayed verbal memory. Some researchers have tested immediate and delayed word recall in glucose loading, GI and GL studies (Draeos et al., 1995; Dye et al., 2010; Foster, Lidder, & Sünram, 1998; Micha et al., 2010). Conflicting results have justified the need for further testing under controlled conditions.

The wordlists used in this study (Appendix C) were adapted from Hopkins Verbal Learning Test which is standardised, validated and repeatable (Brandt, 1991). Hopkins wordlists contain 12 words to recall for each test with words from three semantic categories. The lists were increased to contain twenty words to make the test more difficult as the list is repeated in each testing period. The three semantic categories were increased to contain six words in each category, and two distractor words were added that belonged to none of the semantic categories.

Six wordlists were available. One wordlist was used per session. The remaining wordlist was used for a practice run of the tests in lectures (to be discussed in 4.4.4).

Free Word Recall Test

Audible versions of the wordlists spoken by a computer-generated voice were played to participants over the laboratory speakers. The words were played at a

frequency of one word per second. The words were listed in randomised order, and the order of words were altered each time the list was played ensuring the first and last word of the list were always different.

To increase the cognitive demand of the free word recall test, tests were performed under the condition of divided attention. To do this, participants performed motor sequences while they heard the wordlists (Foster et al., 1998). The candidate created different hand motor sequences for each free word test so the distraction exercise was unfamiliar each time. The motor sequences consisted of three actions eg. OKAY-WAVE-DROP. The participants learned the motor sequence in successive order and reverse order eg. LIFT-BACKWAVE-OKAY. They were instructed to swap between each motor sequence after every five words they heard. While the wordlists were played aloud, the motor sequence description was displayed on the screens.

Immediately after the wordlist was played, participants had 45 seconds to write down all of the words they could recall in no particular order.

Short Delay and Long Delay Word Recall Test

Five minutes and twenty minutes after the completion of the free word recall test, participants had 45 seconds to write down as many of the words they could recall from that test.

Letter-Number Sequence Recall Test

Letter number sequencing tests working memory (Diamond, 2013). Working memory differs from short term memory whereby short term memory involves holding information in mind whereas working memory involves holding information in mind (in letter number sequencing this is a series of letters and numbers) and mentally arranging that information (Diamond, 2013).

Eight different sequences were presented. The sequences increased in length by one letter or digit each time beginning from three characters long. The sequences were generated using the randomise function on Microsoft Excel (version 15.32).

For consistency of difficulty, sequences were checked and improved by the candidate to ensure that: none of the sequences spelt any commonly known words, sounds or abbreviations, sequences did not have any two digits beside one another, sequences with six or more characters followed a letter number pattern e.g. a six letter (L) number (N) sequence followed the order of N.L.L.N.L.L.

Each sequence was displayed on screen for five seconds followed by a ten second gap for participants to write their recollection of the sequence down.

Visuospatial Recall Test

The visuospatial test was designed by the candidate as repeatable tests were required that could be administered using a screen for display with answers on paper.

For each test, a picture was displayed for ten seconds followed by a blank screen for three seconds. Five questions were asked about the picture. Each question displayed for twenty seconds. The questions were either multiple choice or required the count of an object.

The pictures were made using online design programme Canva. An example of the tests is supplied in (Appendix D). Different tests were created to minimise any learning effect that could be caused if a participant saw the same picture and questions each time. To make the test different but equivalent in difficulty to one another all the pictures looked similar at a glance and the pictures and questions were designed with strict criteria:

- All pictures had thirty objects on a two-tone landscape background;
- A pool of twenty-three objects were selected from for use in each picture;

- All pictures contained mountains, buildings, trees, flowers, animals, a sun or moon and a person speaking a percentage in a speech bubble;
- The five questions asked about the picture were the same at each session for the tests at the same time point but in a randomised order. Participants were not told that the questions were the same, and as the two test days' participants attended were two-three weeks apart, it was deemed unlikely they would recall the questions asked or at which time point they were presented in;
- The number of multiple choice answers to the same question in all tests from that time-point were the same;
- The incorrect answers were made using the same method for each corresponding test, e.g. on the question "Which tree did you see?", an incorrect multiple choice answer had the same shape tree, with the colour of the sky and the colour of the duck's body in the memorised picture in all baseline tests.

Reitan's Trail Making Part B

This trail test measures visual attention, task switching, and speed of processing (Broshek & Barth, 2000; Salthouse, 2011).

The task involves joining dots containing letters and numbers in alternate, ascending order e.g. '1-A-2-B-3-C' etc. until the final letter 'L'. Participants must not remove their pen from the paper until they have completed the test. Performance is measured by the time it takes an individual to complete.

The participants performed this test once at each session. To reduce learning effects to the pattern of the trail, two versions of the trail test part B were used, the original and a mirror image of the original. To control for order, half of order 1 and order 2 were randomly assigned to perform the original test first followed by the mirror image. The other half were assigned to perform the tests in the reverse order.

Laboratory facilitators timed each participant individually from beginning to completion of the test.

4.4 Research Procedures

4.4.1 Randomisation

Participants were computer randomised to the order in which they received each trifle. Thirty-nine participants were randomised to receive the sucrose-sweetened trifle first, and thirty-eight the isomaltulose-sweetened trifle first. Equal sex distribution was achieved by block randomising the sexes to control for the potential source of variability. Participants were randomly scheduled to two research sessions with a two- or three-week gap between them.

4.4.2 Double Blinding

A staff member of the Department of Human Nutrition labelled the trifles with a red or green sticker such that the study investigators and the participants were blinded to treatment.

The trifles were visually indistinguishable and served in identical containers.

4.4.3 Data Collection

Participants attended two sessions, two or three-weeks apart, on Fridays at 12:00pm – 3:30 pm. A total of five sessions were held in an attempt to accommodate all participants. The sessions were held at Mellor Laboratories, University Otago.

4.4.4 Preparatory Procedures

An information sheet outlining the study protocol was provided to the participants who were given the opportunity to clarify any queries they may have had with the investigators.

The participants were given a practice run of the free word recall and visuospatial tests for familiarisation of the format to reduce learning effects. The participants were also made aware of the other tests that would be performed in the laboratories but prior familiarisation was not necessary.

4.4.5 Demographics Collection

The participants completed a demographics and dietary restriction questionnaire at their first session (Appendix E). Information was collected on colour blindness and whether English was the participant's first language, as these factors could have affected participant scores in the cognitive tests.

During the sessions, measures of body weight and height were taken by a research assistant trained in anthropometry measurement. Body weight was measured using a Seca alpha 770 digital scale (Seca, Hamburg, Germany), accurate to 0.1 kg. Height was measured using a Holtain stadiometer (Holtain limited, Dyfed, West Germany), accurate to 0.01 cm. Using these measures, body mass index (BMI) was calculated by dividing body weight [kg] by the square of the height [m²].

4.4.6 Testing Day Procedure

For standardisation, participants were provided with breakfast cereal to take home and to eat on the morning of each test day. Participants could choose the cereal and the amount. Participants were at liberty to add anything to their cereal but were requested to do the same on both testing days. Participants were requested to fast after breakfast until they received their trifle and after eating their trifle until the testing session ended.

In the first session, participants were provided with information sheets on the study again. Participants consented to participation by signing a consent form that was subsequently signed by the researchers prior to commencing the study (Appendix F).

Capillary blood samples were collected three times throughout each session at baseline and 60 and 120 minutes after participants began eating their trifles (**figure 4.2**).

Tests of cognition were performed three times throughout each session. Baseline cognitive tests were performed to control for differences in test scores irrespective of GI. The remaining cognitive tests were implemented at 60 and 120 minutes after the participants first began eating the trifle.

Participants had 20 minutes to eat their trifle. For comparability, participants who did not eat their entire trifle on their first session, were given the same amount eaten then for their second session.

4.4.7 Blood Collection and Analysis

Capillary blood was obtained by finger pricking using sterilised disposable lancet to collect 500 μ L into a collection tube. Blood samples were used to measure glucose concentration.

A registered nurse trained the candidates and lab facilitators in finger pricking and blood collection and was present during all blood collection sessions.

To aid peripheral blood flow, heated wheat packs were applied to hands for five minutes before blood collection. Fingers were sanitised with alcohol swabs and then pricked with a disposable BD microtainer® contact-activated 2.0 x 1.5mm disposable lancet. Blood was collected into tubes containing 10 μ L of potassium Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Bloods were centrifuged 5-20 minutes after collection for 10 minutes at 2000 RCF. Plasma was then pipetted

into microcentrifuge tubes and stored at -80 degrees Celsius at the University of Otago, Human Nutrition Laboratories, New Zealand for one week to one month before analysis.

Blood glucose concentrations were determined using an enzymatic colorimetric kit on a Cobas c 311 auto-analyser (Roche, Indianapolis, IN, USA). The laboratory adheres to quality control procedures (Westgard rules) and the use of manufacturers controls (Appendix G,H). Intrassay and interassay variations were determined regularly with a pooled plasma sample. Repeatability and accuracy tests were also performed using a Roche commercial control Precinorm U (2 levels). Coefficients of variation were 1.25 %, 0.67 % and 1.87 % for Precinorm U control one and two, and pooled plasma, respectively.

4.5 Statistical Analysis

4.5.1 Sample Size Estimation

Based on published data a sample size of 60 was sufficient to detect a difference of 0.5 SD for all outcomes in standardized form, with 90% power at the significance level of $P < 0.01$.

4.5.2 Statistical Analysis

Mixed-effects regression analysis was used to analyse the effects the sugars had on cognitive test scores by comparing sucrose to isomaltulose at 60 and 120 minutes. The data were adjusted for English as a second language, special diet, baseline score (no baseline for trail test), and order. Only participants with complete data were included. Participants who did not eat the same amount of trifle at each session were excluded.

Residuals of all models were plotted and visually assessed for homogeneity of variance and normality.

5. Results

Data from 65 participants were included in the analysis. The randomisation, allocation and exclusion of participants can be viewed in **figure 5.1**. Participants who did not take part in both testing days, or did not eat all of their trifle on the second testing day were excluded from the analysis.

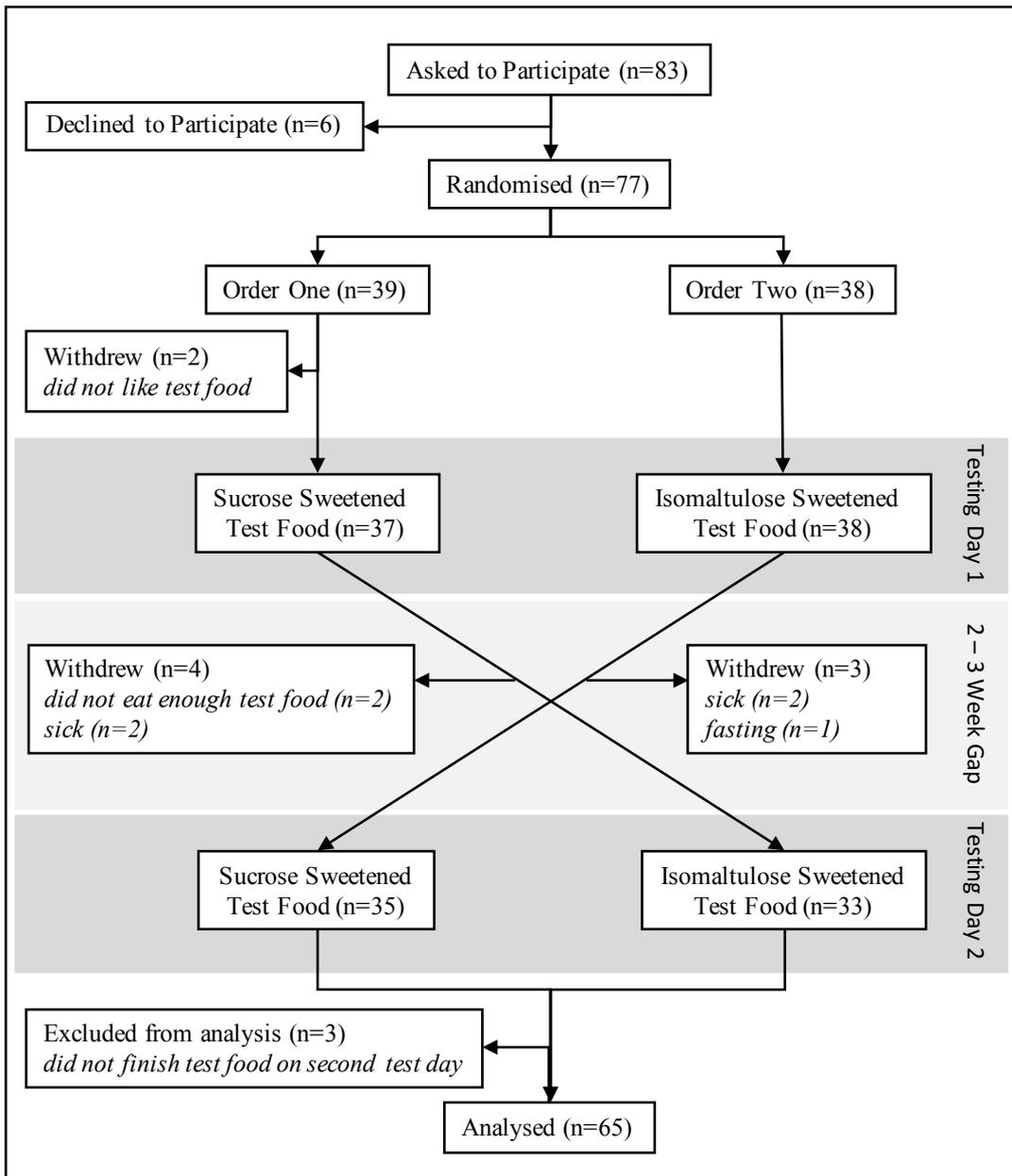


Figure 5.1 Study Design and Participant Flow Diagram

The demographic characteristics of participants are presented in **Table 5.1**. The study sample had a high ratio of females to males. Most participants were NZ European and in their early twenties. None of the participants had been diagnosed with diabetes. No-one was colour blind so all complete visuospatial tests were used in the analysis.

Table 5.1 Demographic Characteristics of Participants

Characteristics	Participants (n=65)
Age (y)^a	21.9 (5.8)
Gender (n)[*]	
Female	57 (88)
Male	8 (12)
Weight (kg)^a	65.97 (13.3)
Height (m)^a	1.66 (0.1)
BMI (kg/m²)^a	23.7 (3.7)
Ethnicity (total response)[*]	
NZ European	40 (62)
Maori	5 (8)
Pacific Island	2 (3)
Asian	17(26)
Other	4 (6)
English not first language[*]	16 (25)

^{*}Results presented as n (%)

^aResults presented as mean (SD)

Order 1 received sucrose then isomaltulose and order received the reverse order.

The blood glucose concentrations of the participants are displayed in **Figure 5.2**. There was no significant difference between the participants baseline blood glucose concentrations (95% CI, -0.28, 0.07 mmol/L). The isomaltulose trifle produced a significantly lower glycaemic response at 60 minutes than the sucrose trifle by -0.69 mmol/L (95% CI, -1.12, -0.25). At 120 minutes, there was no significant difference in concentrations (95% CI, -0.06, 0.52 mmol/L).

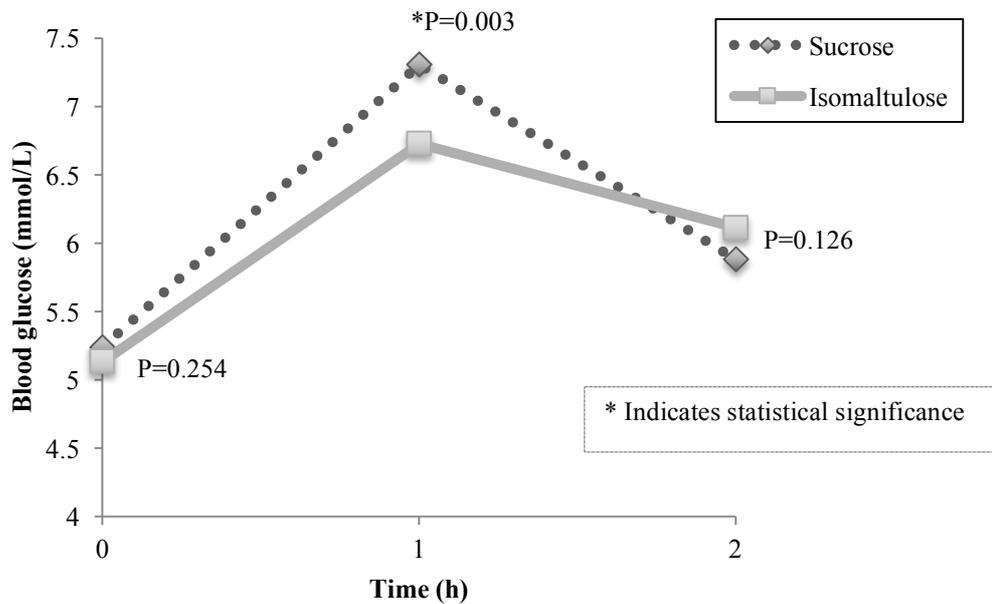


Figure 5.2 Blood glucose concentrations at each cognitive test period

The unadjusted and adjusted models of the cognitive test results were both computed. English as a second language and special diet test foods showed a small influence on the results. Therefore, the model adjusted for English as a second language, special diet test foods, order and baseline scores is presented in **Table 5.2**.

No significant differences in cognitive function after sucrose and isomaltulose were detected for any of the memory tests.

The participants performed faster on the trail test after the sucrose trifle than after the isomaltulose trifle (P=0.037).

Table 5.2 Adjusted mean differences in cognitive test scores between sugars at 60 and 120 minutes*

Test	n	Baseline	60 Minutes		120 Minutes	
		Mean (SD)	Mean difference [†] (95% CI)	P-value	Mean difference (95% CI)	P-value
Free Word Recall	61	6.3 (1.7)	0.0 (-0.6, 0.5)	0.963	0.1 (-0.5, 0.7)	0.686
Short Delay Word Recall	59	5.5 (1.5)	0.0 (-0.5, 0.5)	0.983	-0.3 (-1.0, 0.5)	0.458
Long Delay Word Recall	58	5.7 (1.7)	0.0 (-0.6, 0.6)	0.944	0.2 (-0.6, 0.9)	0.611
Letter Number Sequence Recall	62	4.5 (1.0)	0.3 (-0.2, 0.7)	0.286	0.1 (-0.3, 0.5)	0.576
Visuospatial Recall	63	1.8 (0.8)	-0.2 (-0.6, 0.2)	0.283	0.1 (-0.2, 0.5)	0.440
Trail Test (s)^a	63	50.3 (21.4)	-7.7 (-14.9, -0.5)	0.037		

[†]All differences are sucrose compared to isomaltulose.

*Adjusted for English as a second language, special diet, baseline score (except for trail test) and order.

^aNo baseline test was performed, the presented mean (SD) baseline score is the 60-minute score from the first laboratory participants attended.

6. Discussion

The primary aim of this study was to determine if the postprandial glycaemic response influences cognitive function. Although a difference in postprandial glycaemia was generated by incorporating two sugars with very different GIs (sucrose GI = 65, and isomaltulose GI = 34) into trifle, there was no difference in cognitive test scores between trifles for memory-related tests. The time to complete a trail-making test one hour after trifle consumption was significantly less following the higher GI trifle.

Our findings both correspond with, and contradict, findings from past research. Heterogeneity in findings across studies is perhaps expected because of the subjective nature of the tests, the variety of tests, bias and confounding. Some findings may have been subjected to bias. This is because investigators and participants were not always blinded by GI category or to the nutrient quality of the meal. Participants may have had pre-existing views on the healthiness of foods and how that may affect cognitive performance and this may have influenced expectations of their ability or motivation to perform a task (Cooper et al., 2015; Micha et al., 2010; Micha et al., 2011). For example, investigators and participants had prior knowledge of breakfasts before administering cognitive tests, a potential source of bias (Micha et al., 2010). In our study, the investigators and participants were both blinded to treatment.

Another prominent issue with past research is confounding due to differences in test meals apart from just GI including food type, energy content, macro- and micro-nutrient content, and/or meal size (Benton et al., 2007; Cooper et al., 2015; Ingwersen, Defeyter, Kennedy, Wesnes, & Scholey, 2007; Micha et al., 2011; Young & Benton, 2014a, 2014b). In an observational study, every participant had a different meal (Micha et al., 2010). Each factor may influence cognitive function, thus it is necessary to

control these variables (Gibson & Green, 2007). In these studies, it is difficult to determine if cognitive function was influenced by GI or by one or a combination of the other varying factors. The test food in our study only differed by the rate of absorption of the sugars, therefore any effect on cognitive function can more confidently be attributed to the GI property of the sugars.

In addition to bias and confounding, the GI or GL of the meals in some of the studies were obtained from published values (Benton et al., 2007; Cooper et al., 2015; Micha et al., 2010; Micha et al., 2011; Young & Benton, 2014a, 2014b). This may be problematic because predicting meal GI by calculation can differ markedly from directly measured GI (Dodd, Williams, Brown, & Venn, 2011). In some studies, additional uncertainty in GI estimations was introduced when, for example, a meal component was not on the database and assumptions as to the foods GI were made (Young & Benton, 2014b). An observational study relied on participant reported breakfast intake by interview using a photographic atlas for meal portions (Micha et al., 2010). Researchers used this information to estimate the GI and GL and subsequently categorised participants to a high or low GI and GL group. There were no statistically significant differences in blood glucose concentrations which suggests the GI of the breakfasts did not differ, thus differences in cognitive performance cannot be attributed to GI. When assessing the relationship between glycaemia and cognition, it is important to accurately confirm the glycaemic potential of the meals. A strength of our work is that the GI of the trifles were determined by an accredited GI testing laboratory and in addition, we tested blood glucose concentrations in our participants to coincide with the time of cognitive testing.

The variability of the methods between studies complicates the comparison of our findings to others. However, our study removed the described confounding and

biasing factors present in other studies, giving confidence in the reliability of our findings. This study, in addition to GI studies that have assessed long-delayed word recall have found no association between GI and performance (Dye et al., 2010; Micha et al., 2010; Micha et al., 2011). Some of the previous studies that have assessed the same immediate memory processes (word and visuospatial recall) have also found no associations between GI and performance within the two-hour postprandial period (Micha et al., 2010; Young & Benton, 2014a). However, in other studies, support for an association has been found (Benton et al., 2007; Micha et al., 2011; Young & Benton, 2014b). Working memory was not associated with GI in the letter-number sequencing task in our study. Findings from GI studies that have assessed this process vary (Dye et al., 2010; Micha et al., 2010; Micha et al., 2011).

All but one of these comparison studies were potentially confounded by meal variability and some were also not blinded and subject to bias. The study by Dye et al. (2010) that was not subject to these confounders, had similar methods to ours although the cognitive tests differed slightly. It was well conducted with a double-blinded cross-over study that supplied a milk beverage that only differed in GI by using sucrose and isomaltulose. The findings of this study were that GI had no association with the measures of immediate and long delayed visual verbal memory which corresponds with the findings of this study. This repetition in finding is suggestive of reliable results.

The significantly improved performance on the trail test after the higher GI trifle could support the theory that tasks of greater cognitive demand are more greatly affected by circulating glucose as the trail test is extremely cognitively demanding (Draeos et al., 1995; Lezak, Howieson, Loring, Hannay, & Fischer, 2004; Smith et al., 2011). As no differences in performance in the other tests were detected it could be considered that the tasks were not demanding enough to be affected by glucose

concentrations. However, as only 0.002 % of the cognitive test scores in this study were full scores, this suggests the tasks were sufficiently cognitively demanding. Therefore, it is more likely that the higher GI has a positive influence when combining the cognitive processes of working memory, speed of processing and cognitive flexibility.

Additional strengths of our study include our gold standard study design that removed confounders present in previous research. We only made within-subject comparisons using a double-blinded, randomised, controlled, crossover trial. Previous studies made between-subject comparisons which is another potential source of confounding (Benton et al., 2007; Cooper et al., 2015; Micha et al., 2010; Micha et al., 2011; Young & Benton, 2014a, 2014b).

With the range of tests in our cognitive test battery, we were able to assess several different cognitive processes that needed further investigation and clarification due to contradicting associations produced from previous studies. We also added to the literature on GI by using the trail test and testing memory under conditions of divided attention.

It was a strength that all of the participants sat their cognitive tests at the same length of time after eating as one another. This was not always the procedure in past studies (Benton et al., 2007; Micha et al., 2010; Micha et al., 2011). It is important to standardise the test times in order to draw conclusions about the influence of GI on cognitive performance as blood glucose levels vary over time.

A limitation of our study was that our test foods were both categorised as low GI. Therefore, we were unable to determine if cognitive performance after a high GI food differs from that after a low GI food. Blood glucose concentrations have been hypothesised to be linearly related to cognitive function (van de Ven et al., 2012). This suggests that a high GI food would cause a greater difference in performance on the trail

test. Further study is necessary to determine if greater differences in GI have an influence on any of these cognitive processes. In this study however, there was an 11 point difference in our trifles which is greater than some of the differences estimated in past studies (Micha et al., 2010; Micha et al., 2011). We were still able to investigate the differences in cognitive performance with this difference. But a greater GI difference may have a different effect.

6.1 Recommendations

We tested cognitive performance 60 and 120 minutes after trifle consumption. But, the 120-minute glycaemic response measured during the GI tests of the trifles demonstrated that the greatest difference in blood glucose concentrations between the trifles was thirty minutes after consumption. Thus, to determine if glycaemia affects cognition, this may have been the best time to investigate this. However in this study, 60 minutes after consumption, the sucrose-sweetened trifle produced a significantly greater blood glucose concentration than the isomaltulose-sweetened trifle. Therefore, we showed that the different blood glucose concentrations produced by the two trifles did not affect performance on the memory tasks differently. Time to complete the trail making test was better with the higher GI trifle, coincident with higher blood glucose at 60 min. It is possible that the difference in glycaemia was causal, but that would need confirmation with repeat testing.

The trifles did not cause a difference in blood-glucose concentrations at the time of the last cognitive test battery. For some high GI foods, glucose concentrations have been found to fall below baseline values at 120 minutes. This effect has been hypothesised to produce a difference in cognitive function at this time point. The reason this did not occur in our study may be because the GI of the sucrose-sweetened trifle was not high enough to cause a decline in blood-glucose concentration below baseline.

Thus, we were unable to investigate the effect of the drop below baseline on cognitive performance.

It could have been valuable to investigate the effect of GI 180 minutes after food consumption as other studies have reported that low GI meals may improve cognitive performance at this point (Young & Benton, 2014a, 2014b). By this time blood glucose should not differ after a low and high GI meal as glycaemia is usually normalised at this point in people with normal glucose tolerance (Blaak et al., 2012). Thus, an effect on cognitive performance at 180 minutes would indicate that GI may have a lasting effect on cognition that is longer than the glycaemic response. It is recommended to investigate this relationship in any future studies.

It is possible the difficulty of some of the tests in our test battery may have varied which could affect the study outcomes. The Reitan's trail test, wordlist and letter number sequencing have previously been standardised and validated but some adjustments were made. The visuospatial test has not been standardised or tested for reliability and validity. However, we randomised the order of treatment which should control for any varying effect.

To further investigate the relationship between GI and working memory, we could have used the serial sevens test. Our working memory test showed GI had no effect on performance, however Dye et al. (2010) found that low baseline scores were associated with better scores on the serial sevens test, a test of working memory, after the higher GI drink. This effect was detected in a post-hoc analysis, thus it was not powered to assess this and it needs further investigation. It is not clear how well controlled this study was for order and if the groups were counterbalanced by low-baseline scores. We could have included this test, pretested the participants, and had an

equal assignment of participants with low baseline scores to further investigate their findings. This could be assessed in a future study.

Further testing on the effects of GI on the trail test is recommended to determine the effects at different time points. If a higher GI trifle positively influences performance after 60 minutes due to higher blood glucose levels, then it is possible that the opposite effect may be detected 120 minutes after consumption if a high GI food causes a rapid decrease in blood glucose level. Due to the learning effects of this test, we did not assess this task in the second post-trifle test battery to avoid the task becoming too easy, which would make detection of differences in performance challenging if the difficulty of a task is related to glucose utilisation in the brain.

6.2 Conclusion

Our findings show that an eleven-point GI difference of a food in the low GI category did not influence immediate, short delayed and long delayed word recall; immediate visuospatial recall; and working memory differently. However, in tasks that combine working memory, speed of processing, and cognitive flexibility higher GI foods may have a positive influence on performance 60 minutes after intake.

7. Application to Dietetic Practice

Dietitians practice evidence based nutrition. The existing evidence on the relationship between GI and cognitive function is conflicting. The findings of this study do not support a relationship between postprandial blood glucose and tests of memory. The trail making test was completed in quicker time following the higher GI trifle, suggestive that blood glucose could influence a task that combined cognitive flexibility with speed of processing. However, the result of a single task completed at a single time-point does not constitute irrefutable evidence in favour of a higher GI food. The suggestion that circulating blood glucose concentrations are directly related to cognitive performance would be of interest to dietitians, but further well-designed studies are needed to establish such a relationship. However, following a high GI diet to improve cognitive performance may not be conducive to good health in other aspects because classifying a diet by GI does not encompass diet quality or quantity.

Diet suggestions that have more convincing evidence should be suggested to people to improve cognitive performance such as having a breakfast and meeting recommended daily intakes by following the Ministry of Health guidelines (Ministry of Health, 2015). This will ensure intake of polyunsaturated fats, and micronutrients that have been associated with cognitive function without risk of detriment to overall health (Gibson & Green, 2007).

Thus, the current position of dietitians on the topic would be that evidence is inconclusive regarding any effect of GI on cognitive performance and that GI should not be promoted as a guide to food choice for people wanting to improve cognitive performance.

7. References

- Augustin, L. S., Franceschi, S., Jenkins, D. J. A., Kendall, C. W. C., & La Vecchia, C. (2002). Glycemic index in chronic disease: a review. *European journal of clinical nutrition.*, 56(11), 1049-1071. doi:10.1038/sj.ejcn.1601454
- Augustin, L. S. A., Kendall, C. W. C., Jenkins, D. J. A., Willett, W. C., Astrup, A., Barclay, A. W., . . . Poli, A. (2015). Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium. *Nutrition, Metabolism and Cardiovascular Diseases*, 25(9), 795-815.
doi:http://dx.doi.org/10.1016/j.numecd.2015.05.005
- Benton, D., Maconie, A., & Williams, C. (2007). The influence of the glycaemic load of breakfast on the behaviour of children in school. *Physiology & Behavior*, 92(4), 717-724. doi:https://doi.org/10.1016/j.physbeh.2007.05.065
- Bjorck, I. (1994). Food properties affecting the digestion and absorption of carbohydrates. *The American journal of clinical nutrition.*, 59.
- Blaak, E. E., Antoine, J. M., Benton, D., Björck, I., Bozzetto, L., Brouns, F., . . . Vinoy, S. (2012). Impact of postprandial glycaemia on health and prevention of disease. *Obesity Reviews*, 13(10), 923-984. doi:10.1111/j.1467-789X.2012.01011.x
- Brand-Miller J. (2016). *The International Glycemic Index (GI) Database*. Retrieved from: <http://ziag4.mmb.usyd.edu.au/>,
<https://researchdata.andis.org.au/international-glycemic-index-gi-database>
- Brand-Miller, J. F.-P., K. Colagiuri, S. . (2003). *The New Glucose Revolution*. New York: Marlowe.
- Broshek, D. K., & Barth, T. B. (2000). Halstead-Reitan Neuropsychological Test Battery. In G. Groth-Marnat (Ed.), *Neuropsychological Assessment in Clinical Practice: A Guide to Test Interpretation and Integration*. New York: John Wiley and Sons.
- Choi, I.-Y., Lee, S.-P., Kim, S.-G., & Gruetter, R. (2001). In vivo measurements of brain glucose transport using the reversible Michaelis–Menten model and simultaneous measurements of cerebral blood flow changes during hypoglycemia. *Journal of cerebral blood flow & metabolism*, 21(6), 653-663.
- Cooper, S. B., Bandelow, S., Nute, M. L., Morris, J. G., & Nevill, M. E. (2015). Breakfast glycaemic index and exercise: combined effects on adolescents' cognition. *Physiology Behaviour*, 139, 104-111.
doi:10.1016/j.physbeh.2014.11.024
- Cryer, P. E. (2017). Physiologic response to hypoglycemia in normal subjects and patients with diabetes mellitus. In D. M. Nathan & J. E. Mulder (Eds.), *In:UpToDate*: Wolters Kluwer.

- Cummings, J. M., J.,. (2012). Carbohydrates. In: Essentials of Human Nutrition. 4th. ed. New York: Oxford University Press, 2012:21-48.
- Dahlqvist, A. (1963). Human intestinal disaccharidases and hereditary disaccharide intolerance. The hydrolysis of sucrose, isomaltose, palatinose (isomaltulose), and a 1,6-alpha-oligosaccharide (isomalto-oligosaccharide) preparation. *The Journal of clinical investigation.*, 42, 556-562.
- De Graaf, R. A., Pan, J. W., Telang, F., Lee, J.-H., Brown, P., Novotny, E. J., . . . Rothman, D. L. (2001). Differentiation of glucose transport in human brain gray and white matter. *Journal of cerebral blood flow & metabolism*, 21(5), 483-492.
- Diamond, A. (2013). Executive functions. *Annual review of psychology*, 64, 135-168.
- Dodd, H., Williams, S., Brown, R., & Venn, B. (2011). Calculating meal glyceimic index by using measured and published food values compared with directly measured meal glyceimic index. *The American journal of clinical nutrition.*, 94(4), 992-996. doi:10.3945/ajcn.111.012138
- Draelos, M. T., Jacobson, A. M., Weinger, K., Widom, B., Ryan, C. M., Finkelstein, D. M., & Simonson, D. C. (1995). Cognitive function in patients with insulin-dependent diabetes mellitus during hyperglycemia and hypoglycemia. *The American Journal of Medicine*, 98(2), 135-144. doi:http://dx.doi.org/10.1016/S0002-9343(99)80397-0
- Du, H., Van Der A, D. L., & Feskens, E. J. M. (2006). Dietary Glycaemic Index. *Acta Cardiologica*, 61(4), 383-397. doi:10.2143/AC.61.4.2017298
- Dye, L., Gilsenan, M. B., Quadt, F., Martens, V. E., Bot, A., Lasikiewicz, N., . . . Lawton, C. (2010). Manipulation of glyceimic response with isomaltulose in a milk-based drink does not affect cognitive performance in healthy adults. *Molecular Nutrition and Food Research*, 54(4), 506-515. doi:10.1002/mnfr.200900196
- Foster, J. K., Lidder, P. G., & Sünram, S. I. (1998). Glucose and memory: fractionation of enhancement effects? *Psychopharmacology*, 137(3), 259-270. doi:10.1007/s002130050619
- Gagne, L. (2008). The glyceimic index and glyceimic load in clinical practice. *Explore*, 4(1), 66-69. doi:10.1016/j.explore.2007.10.011
- Gibson, L. E., & Green, M. W. (2007). Nutritional influences on cognitive function: mechanisms of susceptibility. *Nutrition Research Reviews*, 15(1), 169-206. doi:10.1079/NRR200131
- Glyceimic Index Foundation. (2017). Emerging Science. Retrieved from <http://www.gisymbol.com/emerging-science/>
- Goff, L. M., Cowland, D. E., Hooper, L., & Frost, G. S. (2013). Low glycaemic index diets and blood lipids: A systematic review and meta-analysis of randomised

- controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, 23(1), 1-10. doi:http://dx.doi.org/10.1016/j.numecd.2012.06.002
- Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*, 17(3), 427-437. doi:http://dx.doi.org/10.1016/S0271-5317(97)00010-9
- Graveling, A. J., Deary, I. J., & Frier, B. M. (2013). Acute Hypoglycemia Impairs Executive Cognitive Function in Adults With and Without Type 1 Diabetes. *Diabetes care*, 36(10), 3240.
- Gruetter, R., Ugurbil, K., & Seaquist, E. R. (1998). Steady - State Cerebral Glucose Concentrations and Transport in the Human Brain. *Journal of neurochemistry*, 70(1), 397-408.
- Hasselbalch, S. G. (1994). Brain metabolism during short-term starvation in humans. *Journal of cerebral blood flow & metabolism*, 14(1), 125-131.
- Holmes, R. (1971). Carbohydrate digestion and absorption. *Journal of Clinical Pathology.*, 5, 10.
- Holub, I., Gostner, A., Theis, S., Nosek, L., Kudlich, T., Melcher, R., & Scheppach, W. (2010). Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose). *British Journal of Nutrition*, 103(12), 1730-1737.
- Ingwersen, J., Defeyter, M. A., Kennedy, D. O., Wesnes, K. A., & Scholey, A. B. (2007). A low glycaemic index breakfast cereal preferentially prevents children's cognitive performance from declining throughout the morning. *Appetite*, 49(1), 240-244. doi:http://dx.doi.org/10.1016/j.appet.2006.06.009
- Inkster, B., & Frier, B. M. (2012). The effects of acute hypoglycaemia on cognitive function in type 1 diabetes. *The British Journal of Diabetes & Vascular Disease*, 12(5), 221-226. doi:doi:10.1177/1474651412466273
- Jenkins, D. J. A. (1981). Glycemic index of foods: A physiological basis for carbohydrate exchange. *The American journal of clinical nutrition.*, 34(3), 362-366.
- Jenkins, D. J. A., Ghafari, H., Wolever, T. M. S., Taylor, R. H., Jenkins, A. L., Barker, H. M., . . . Bowling, A. C. (1982). Relationship between rate of digestion of foods and post-prandial glycaemia. *Diabetologia*, 22(6), 450-455. doi:10.1007/BF00282589
- Kashimura, J., Nagai, Y., & Ebashi, T. (2003). The effect of palatinose on mental concentration in humans. *Journal of nutritional science and vitaminology*, 49(3), 214-216.
- Laterra, J. K., Betz, R., Lorriss, A., & Goldstein, G. W. (1999). Basic Neurochemistry: Molecular, Cellular and Medical Aspects. In G. J. Siegel, B. W. Agranoff, S. K.

- F. R. W. Albers, & M. D. Uhler (Eds.), *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. Philadelphia: Lippincott-Raven.
- Lezak, M. D., Howieson, D. B., Loring, D. W., Hannay, H. J., & Fischer, J. S. (2004). *The Neuropsychological Evaluation* (4 ed.). New York: Oxford University Press.
- Lina, B. A. R., Jonker, D., & Koziarowski, G. (2002). Isomaltulose (Palatinose®): a review of biological and toxicological studies. *Food and Chemical Toxicology*, *40*(10), 1375-1381. doi:http://dx.doi.org/10.1016/S0278-6915(02)00105-9
- Liu, J., Hwang, W.-T., Dickerman, B., & Compher, C. (2013). Regular breakfast consumption is associated with increased IQ in kindergarten children. *Early human development*, *89*(4), 257-262. doi:10.1016/j.earlhumdev.2013.01.006
- Livesey, G., Taylor, R., Hulshof, T., & Howlett, J. (2008). Glycemic response and health—a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. *The American journal of clinical nutrition*, *87*(1), 258S-268S.
- Ludwig, D. S. (2002). The glycemic index: Physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *The Journal of the American Medical Association*, *287*(18), 2414-2423.
- Lund-Andersen, H. (1979). Transport of glucose from blood to brain. *Physiological reviews*, *59*(2), 305-352.
- Maeda, A., Miyagawa, J., Miuchi, M., Nagai, E., Konishi, K., Matsuo, T., . . . Murai, K. (2013). Effects of the naturally - occurring disaccharides, palatinose and sucrose, on incretin secretion in healthy non - obese subjects. *Journal of diabetes investigation*, *4*(3), 281-286.
- Manning, C. A., Parsons, M. W., Cotter, E. M., & Gold, P. E. (1997). Glucose effects on declarative and nondeclarative memory in healthy elderly and young adults. *Psychobiology*, *25*(2), 103-108.
- Manning, C. A., Ragozzino, M. E., & Gold, P. E. (1993). Glucose enhancement of memory in patients with probable senile dementia of the Alzheimer's type. *Neurobiology of aging*, *14*(6), 523-528.
- Manning, R. (1982). The serial sevens test. *Archives of Internal Medicine*, *142*(6), 1192. doi:10.1001/archinte.1982.00340190148022
- Micha, R., Rogers, P., & Nelson, M. (2010). The glycaemic potency of breakfast and cognitive function in school children. *European journal of clinical nutrition*, *64*(9), 948-957.
- Micha, R., Rogers, P. J., & Nelson, M. (2011). Glycaemic index and glycaemic load of breakfast predict cognitive function and mood in school children: a randomised controlled trial. *British Journal of Nutrition*, *106*(10), 1552-1561. doi:10.1017/S0007114511002303

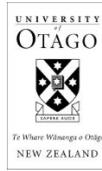
- Ministry of Health. (2015). Current Food and Nutrition Guidelines. Retrieved from <https://www.health.govt.nz/our-work/eating-and-activity-guidelines/current-food-and-nutrition-guidelines>
- Mulholland, H., Murray, L., Cardwell, C., & Cantwell, M. (2008). Dietary glycaemic index, glycaemic load and breast cancer risk: a systematic review and meta-analysis. *British journal of cancer*, 99(7), 1170-1175.
- Philippou, E., & Constantinou, M. (2014). The Influence of Glycemic Index on Cognitive Functioning: A Systematic Review of the Evidence. *Advances in Nutrition*, 5(2), 119-130. doi:10.3945/an.113.004960
- Pollitt, E. (1995). Does Breakfast Make a Difference in School? *Journal of the American Dietetic Association*, 95(10), 1134-1139. doi:[http://dx.doi.org/10.1016/S0002-8223\(95\)00306-1](http://dx.doi.org/10.1016/S0002-8223(95)00306-1)
- Pollitt, E., Lewis, N. L., Garza, C., & Shulman, R. J. (1982). Fasting and cognitive function. *Journal of Psychiatric Research*, 17(2), 169-174. doi:[https://doi.org/10.1016/0022-3956\(82\)90018-8](https://doi.org/10.1016/0022-3956(82)90018-8)
- PubChem Compound Database. Compound Summary for Palatinose, CID=439559. National Center for Biotechnology Information. . [https://pubchem.ncbi.nlm.nih.gov/compound/Palatinose - section=Top](https://pubchem.ncbi.nlm.nih.gov/compound/Palatinose-section=Top)
- PubChem Compound Database. Compound Summary for Sucrose, CID 5988. National Center for Biotechnology Information. .
- S. Sivakumaran, & L Huffman, S. S. (2017). *The Concise New Zealand Food Composition Tables*. Retrieved from Palmerston North, New Zealand: <http://www.foodcomposition.co.nz/>
- Salmerón, J., Manson, J. E., Stampfer, M. J., Colditz, G. A., Wing, A. L., & Willett, W. C. (1997). Dietary fiber, glycemic load, and risk of non—insulin-dependent diabetes mellitus in women. *JAMA*, 277(6), 472-477. doi:10.1001/jama.1997.03540300040031
- Salthouse, T. A. (2011). What cognitive abilities are involved in trail-making performance? *Intelligence*, 39(4), 222-232. doi:<https://doi.org/10.1016/j.intell.2011.03.001>
- Scholey, A. B., Harper, S., & Kennedy, D. O. (2001). Cognitive demand and blood glucose. *Physiology & Behavior*, 73(4), 585-592. doi:[https://doi.org/10.1016/S0031-9384\(01\)00476-0](https://doi.org/10.1016/S0031-9384(01)00476-0)
- Simpson, I. A., Carruthers, A., & Vannucci, S. J. (2007). Supply and Demand in Cerebral Energy Metabolism: The Role of Nutrient Transporters. *Journal of cerebral blood flow & metabolism*, 27(11), 1766-1791. doi:doi:10.1038/sj.jcbfm.9600521
- Smith, M. A., Riby, L. M., Eekelen, J. A. M. v., & Foster, J. K. (2011). Glucose enhancement of human memory: A comprehensive research review of the

- glucose memory facilitation effect. *Neuroscience & Biobehavioral Reviews*, 35(3), 770-783. doi:<https://doi.org/10.1016/j.neubiorev.2010.09.008>
- Sun, S. Z., & Empie, M. W. (2012). Fructose metabolism in humans – what isotopic tracer studies tell us. *Nutrition & Metabolism*, 9, 89. doi:10.1186/1743-7075-9-89
- Sünram-Lea, S. I. O., Lauren. Robertson, Bernadette. (2015). Glycaemic Control and Cognition: Evidence across the Lifespan. In T. D. Best, Louise. (Ed.), *Nutrition for Brain Health and Cognitive Performance*: CRC Press.
- Thomas, D., Elliott, E. J., & Baur, L. (2007). Low glycaemic index or low glycaemic load diets for overweight and obesity. *The Cochrane Library*.
- University of Otago and Ministry of Health. (2011). *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.
- van de Ven, K. C. C., van der Graaf, M., Tack, C. J., Heerschap, A., & de Galan, B. E. (2012). Steady-State Brain Glucose Concentrations During Hypoglycemia in Healthy Humans and Patients With Type 1 Diabetes. *Diabetes*, 61(8), 1974-1977. doi:10.2337/db11-1778
- Welch, I. M., Bruce, C., Hill, S., & Read, N. (1987). Duodenal and ileal lipid suppresses postprandial blood glucose and insulin responses in man: possible implications for the dietary management of diabetes mellitus. *Clinical Science*, 72(2), 209-216.
- Wolever, T. M. S. (1991). The glycemic index: Methodology and clinical implications. *The American journal of clinical nutrition.*, 54(5), 846-854.
- Young, H., & Benton, D. (2014a). The effect of using isomaltulose (Palatinose™) to modulate the glycaemic properties of breakfast on the cognitive performance of children. *European Journal of Nutrition*, 54(6), 1013-1020.
- Young, H., & Benton, D. (2014b). The glycemic load of meals, cognition and mood in middle and older aged adults with differences in glucose tolerance: A randomized trial. *e-SPEN Journal*, 9(4), e147-e154. doi:10.1016/j.clnme.2014.04.003
- Zhu, Y., Hsu, W. H., & Hollis, J. H. (2013). The Impact of Food Viscosity on Eating Rate, Subjective Appetite, Glycemic Response and Gastric Emptying Rate. *PLoS ONE*, 8(6), e67482. doi:10.1371/journal.pone.0067482

8. Appendices

Appendix A: Ethics Proposal and Approval Letter	52
Appendix B: Trifle Recipe and Nutrient Information	54
Appendix C: Wordlists for Free Word, Short Delay and Long Delay Recall Tests.....	55
Appendix D: Visuospatial Test Example.....	56
Appendix E: Participant Demographics Form.....	59
Appendix F: Consent Form	60
Appendix G: Westgard Rules	62
Appendix H: Roche Cobas C Glucose Analysis Procedure	66

Appendix A: Ethics Proposal and Approval Letter



H17/011

Academic Services
Manager, Academic Committees, Mr Gary Witte

8 February 2017

Dr B Venn
Department of Human Nutrition
Division of Sciences

Dear Dr Venn,

I am again writing to you concerning your proposal entitled "**HUNT311 clinical nutritional laboratory; a repeated teaching activity**", Ethics Committee reference number **H17/011**.

Thank you for your e-mail of 7th February 2017, with attached documentation, addressing the issues raised by the Committee.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

<http://www.otago.ac.nz/healthandsafety/index.html>

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office:

gary.witte@otago.ac.nz

jo.farronediaz@otago.ac.nz

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval or an extension of approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

The Human Ethics Committee (Health) asks for a Final Report to be provided upon completion of the study. The Final Report template can be found on the Human Ethics Web Page <http://www.otago.ac.nz/council/committees/committees/HumanEthicsCommittees.html>

Yours sincerely,



Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor S Samman Department of Human Nutrition

Appendix B: Trifle Recipe and Nutrient Information

Serves 20

Sponge

10 g	eggs	Beat eggs and sugar until creamy. Fold flours and BP in and bake with tinfoil at 190 ° C on fan bake.
690 g	sucrose or isomaltulose	
325 g	flour	
125 g	corn flour	
10 g	baking powder	

Jelly

2200 g	water	In a saucepan, cover and bring water and sugar to boil, boil for 1 minute. Whisk together gelatine and lemon juice. When syrup has boiled whisk it in to the gelatine mix. Add the extra water, stir well once more to combine. Cover.
625 g	sucrose or isomaltulose	
625 g	lemon juice	
75 g	gelatine	
500 g	water	

Custard

2850 g	milk	In a double boiler, bring the milk, and cream to 88° C slowly over a low heat. Whisk the yolks, sugar and cornflour together in a bowl until well blended. Pour the hot milk and cream on to the eggs and sugar, whisking all the time with a balloon whisk. Return to double boiler, add vanilla extract, and over a low heat gently stir with a wooden spatula at 88° C until thickened.
275 g	cream	
2.5 tsp	vanilla essence	
20 g	egg yolks	
150 g	sucrose or isomaltulose	
30 g	cornflour	

Trifle Nutrient Information	
Serving Size: 446g	
	Quantity per serving
Energy	2517.6 kJ
Protein	16.1 g
Total Fat	15.3 g
Total Available Carbohydrate	98.8 g

Appendix C: Wordlists for Free Word, Short Delay and Long Delay Recall Tests

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
Semantic Group 1	Diamond	Rifle	Vanilla	Socks	Spinach
	Sapphire	Gun	Cinnamon	Skirt	Carrot
	Ruby	Pistol	Sugar	Shoes	Potato
	Pearl	Bomb	Garlic	Pants	Lettuce
	Opal	Sword	Salt	Blouse	Bean
	Emerald	Arrow	Chilli	Shirt	Corn
Semantic Group 2	Hut	Knife	Oil	Canary	Tennis
	Cave	Pan	Diesel	Robin	Golf
	House	Pot	Kerosene	Eagle	Soccer
	Apartment	Spoon	Gasoline	Sparrow	Baseball
	Hotel	Spatula	Electricity	Bluebird	Basketball
	Tent	Fork	Coal	Crow	Football
Semantic Group 3	Horse	Wine	Trumpet	Saw	Engineer
	Cow	Rum	Harmonica	Nails	Doctor
	Lion	Beer	Violin	Wrench	Dentist
	Tiger	Whiskey	Drum	Screwdriver	Teacher
	Wolf	Bourbon	Flute	Hammer	Professor
	Cat	Vodka	Clarinet	Chisel	Lawyer
Distractor Words	Tractor	Ocean	Pug	Cola	Rice
	Pencil	Foot	Father	Acorn	Tablet

Appendix D: Visuospatial Test Example



Q1: Which tree did you see?



A



B



C



D

Q2. What building did you see?



A



B

This building was not in the picture

E

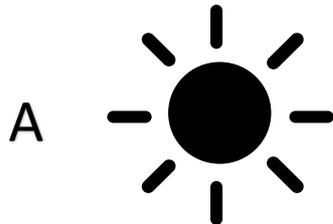


C



D

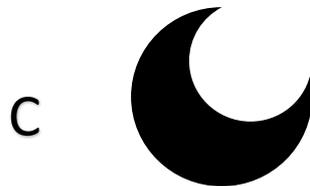
Q3: What shape was in the sky?



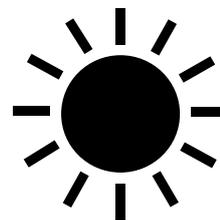
A



B



C



D

Q4: Which animal did you see?



A



B



C



D

Q5: How many clouds were in the sky?

Appendix E: Participant Demographics Form

Clinical Nutrition Laboratory Demographics Sheet

The information below will help us better understand the group results. This information is voluntary, if you do not wish to answer any question you may move to the next question. The information you provide will be de-identified and pooled with the results of every other participant to describe the group.

Student ID: _____

Lab ID Number: _____

Date of Birth: _____

Male / Female (please circle)

Have your weight and height taken during this laboratory session

Lab facilitator to fill out this section

Weight: _____ kg

Height: _____ m

Which ethnic group do you belong to? Please tick the box or boxes that apply to you.

- New Zealand European
- Māori
- Pacific Island
- Asian
- Indian
- Other – Please specify: _____

Is English your first language? Yes / No

Are you colour blind? Yes / No

Have you been diagnosed with diabetes? Yes / No

Please list any food allergy or intolerance:

(please speak to Bernard, Fiona or Olivia if you do and haven't let us know already)

Appendix F: Consent Form

University of Otago Human Ethics Committee (Health)



HUNT311 clinical nutritional laboratory; a repeated teaching activity

Principal Investigator: Dr Bernard Venn (bernard.venn@otago.ac.nz tel 034795068)

CONSENT FORM FOR PARTICIPANTS

Following signature and return to the research team this form will be stored in a secure place for ten years.

Name of participant:.....

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that I meet the criteria for participation which are explained in the Information Sheet.
4. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
6. I know that as a participant I will be asked to provide demographic data and have my height and weight measured. I will provide blood samples via fingerprick and participate in tests of cognition.
7. I know that the laboratory will explore the effect of consuming trifle sweetened with sucrose or isomaltulose on blood glucose, satiety and cognition. If I feel hesitant or uncomfortable I may decline to answer any particular question(s) , and /or may withdraw from the project without disadvantage of any kind.
8. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.
9. I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at least ten years.
10. I understand that the results of the project may be published and be available in the University of Otago Library, but that either (i) I agree that any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study

11. I know that there is no remuneration offered for this study, and that no commercial use will be made of the data.

12. I understand that the blood samples will be disposed of with opportunity to ask for karakia (please indicate preference).

Dispose blood samples in the standard way

Dispose blood samples with a karakia

Signature of participant:

Date:

Name of person taking consent

Date:

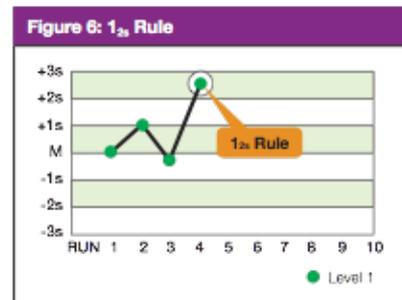
]

Westgard Rules

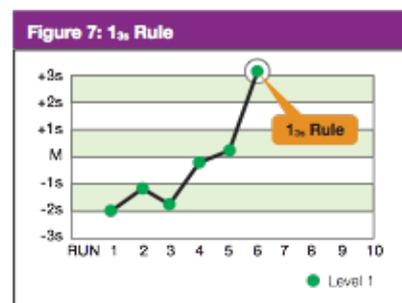
In 1981, Dr. James Westgard of the University of Wisconsin published an article on laboratory quality control that set the basis for evaluating analytical run quality for medical laboratories. The elements of the Westgard system are based on principles of statistical process control used in industry nationwide since the 1950s.¹⁰ There are six basic rules in the Westgard scheme. These rules are used individually or in combination to evaluate the quality of analytical runs.

Westgard devised a shorthand notation for expressing quality control rules. Most of the quality control rules can be expressed as N_L , where N represents the number of control observations to be evaluated and L represents the statistical limit for evaluating the control observations. Thus 1_{2s} represents a control rule that is violated when one control observation exceeds the $\pm 2s$ control limits.

RULE
1_{2s} This is a warning rule that is violated when a single control observation is outside the $\pm 2s$ limits. Remember that in the absence of added analytical error, about 4.5% of all quality control results will fall between the 2s and 3s limits. This rule merely warns that random error or systematic error may be present in the test system. The relationship between this value and other control results within the current and previous analytical runs must be examined. If no relationship can be found and no source of error can be identified, it must be assumed that a single control value outside the $\pm 2s$ limits is an acceptable random error. Patient results can be reported.



RULE
1_{3s} This rule identifies unacceptable random error or possibly the beginning of a large systematic error. Any QC result outside $\pm 3s$ violates this rule.



¹⁰ There are several laboratory QC software packages that use the Westgard scheme. Unity Real Time™ software from Bio-Rad Laboratories is one such package. It not only uses the basic six rules, but unlike other laboratory QC software packages, it also uses additional applications for evaluation of run quality. The Westgard rules can be used manually in concert with Levy-Jennings charts, but manual application is less efficient.

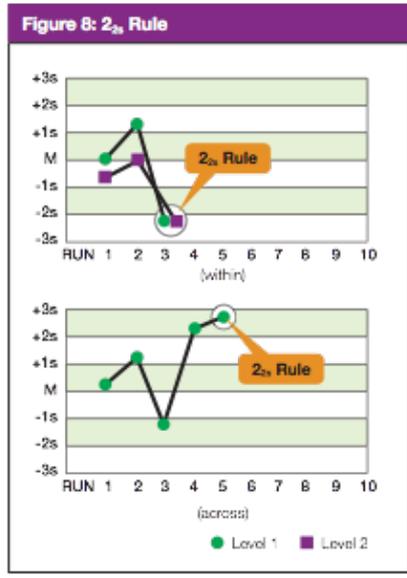
RULE This rule identifies systematic error only. The criteria for violation of this rule are:

2_{2s}

- Two consecutive QC results
- Greater than 2s
- On the same side of the mean

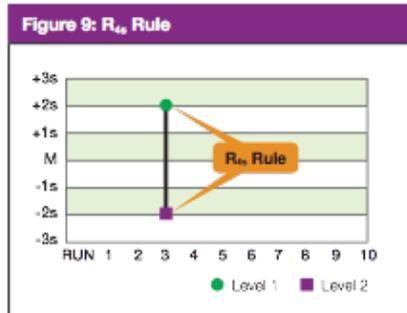
There are two applications to this rule: within-run and across runs. The within-run application affects all control results obtained for the current analytical run. For example, if a normal (Level I) and abnormal (Level II) control are assayed in this run and both levels of control are greater than 2s on the same side of the mean, this run violates the within-run application for systematic error. If however, Level I is -1s and Level II is +2.5s (a violation of the 1_{2s} rule), the Level II result from the previous run must be examined. If Level II in the previous run was at +2.0s or greater, then the across run application for systematic error is violated.

Violation of the within-run application indicates that systematic error is present and that it affects potentially the entire analytical curve. Violation of the across run application indicates that only a single portion of the analytical curve is affected by the error.¹¹



RULE This rule identifies random error only, and is applied only within the current run. If there is at least a 4s difference between control values within a single run, the rule is violated for random error. For example, assume both Level I and Level II have been assayed within the current run. Level I is +2.8s above the mean and Level II is -1.3s below the mean. The total difference between the two control levels is greater than 4s (e.g., [+2.8s - (-1.3s)] = 4.1s).

R_{4s}



¹¹ This rule also applies to 3-level (three level) controls. Whenever any two of the three levels violate the criteria for this rule within the run, unacceptable systematic error may be present and must be resolved.

Violation of any of the following rules does not necessarily require rejection of the analytical run. These violations typically identify smaller systematic error or analytical bias that is not often clinically significant or relevant. Analytical bias may be eliminated by performing calibration or instrument maintenance.

RULE The criteria which must be met to violate this rule are:

3_{1s}

- Three consecutive results
- Greater than 1s
- On the same side of the mean

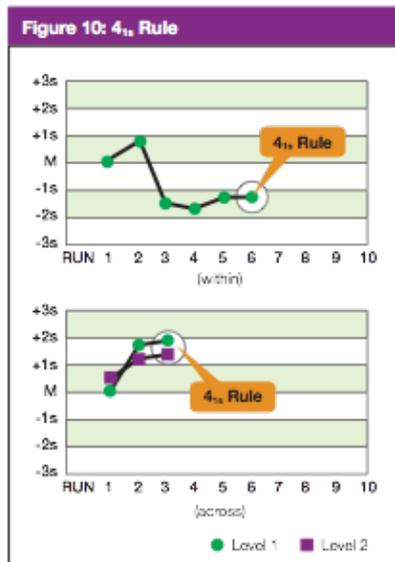
RULE The criteria which must be met to violate this rule are:

4_{1s}

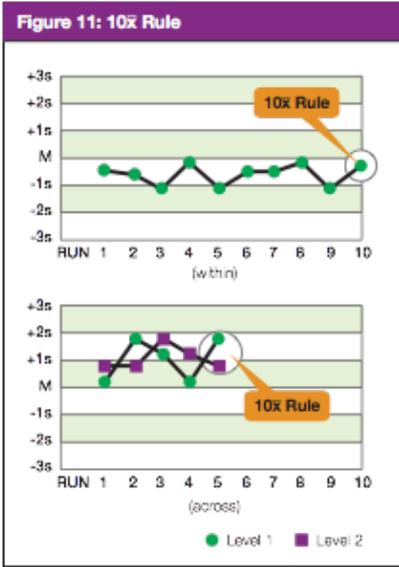
- Four consecutive results
- Greater than 1s
- On the same side of the mean

There are two applications to the 3_{1s} and 4_{1s} rule. These are within control material (e.g. all Level I control results) or across control materials (e.g., Level I, II, and III control results in combination). Within control material violations indicate systematic bias in a single area of the method curve while violation of the across control materials application indicates systematic error over a broader concentration.¹²

Figure 10: 4_{1s} Rule



¹² Use of 3_{1s} detects smaller analytical bias than 4_{1s} and is said to be more sensitive to analytical bias.



RULES

7x | 8x | 9x | 10x | 12x

These rules are violated when there are:

- 7 or 8, or 9, or 10, or 12 control results
- On the same side of the mean regardless of the specific standard deviation in which they are located.

Each of these rules also has two applications: within control material (e.g., all Level I control results) or across control materials (e.g. Level I, II, and III control results in combination). Within control material violations indicate systematic bias in a single area of the method curve while violation of the across control materials application indicates systematic bias over a broader concentration.^{13,14}

¹³ The 7x control rule is far more sensitive to analytical bias than the 12x and the chances of finding seven consecutive control observations on one side of the mean are much higher than finding twelve. It is extremely important that each individual laboratory be aware of highly sensitive rules like 7x, 8x and 9x and apply them sparingly, if at all.

¹⁴ When evaluating different laboratory QC software packages be sure that all applications of the Westgard rules are included. Be careful of instrument QC packages. These may be deficient. Some do not check all six of the Westgard rules or perform both within and between run checking. Refer to the instrument manual or ask the manufacturer about control rule applications for specific instrument models.

Appendix H: Roche Cobas C Glucose Analysis Procedure

GLUC3 Glucose HK



the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability (no hemolysis): ⁵	8 hours at 15-25 °C
	72 hours at 2-8 °C
Stability in fluoride plasma: ⁶	3 days at 15-25 °C

Urine

Collect urine in a dark bottle. For 24-hour urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40 % of their glucose after 24-hour storage at room temperature.³ Therefore, keep samples on ice during collection.⁵

CSF

Cerebrospinal fluid may be contaminated with bacteria and often contains other cellular constituents. CSF samples should therefore be analyzed for glucose immediately or stored at 4 °C or -20 °C.^{3,5}

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section.

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, plasma, urine and CSF

cobas c 311 test definition

Assay type	2 Point End		
Reaction time / Assay points	10 / 6-32 (STAT 7 / 6-32)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H ₂ O)	
R1	28 µL	141 µL	
R2	10 µL	20 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	-	-
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	-	-

cobas c 501/502 test definition

Assay type	2 Point End		
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL, g/L)		
Reagent pipetting		Diluent (H ₂ O)	
R1	28 µL	141 µL	
R2	10 µL	20 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	-	-
Decreased	10 µL	15 µL	135 µL
Increased	4 µL	-	-

Calibration

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration
	- after reagent lot change
	- as required following quality control procedures

Traceability: This method has been standardized against ID/MS.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 18.02 = mg/dL
mmol/L x 0.1802 = g/L
mg/dL x 0.0555 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a glucose concentration of 3.9 mmol/L (70.3 mg/dL).

Serum/plasma

Icterus:⁷ No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:⁷ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid):⁷ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{8,9}

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.⁹

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3 % positive bias on average.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator's manual.

cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

Serum, plasma, urine and CSF

0.11-41.6 mmol/L (2-750 mg/dL)

Determine samples having higher concentrations via the rerun function.

Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.

GLUC3

Glucose HK

cobas®

Lower limits of measurement**Lower detection limit of the test**

0.11 mmol/L (2 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values**Plasma¹⁰**

Fasting 4.11-6.05 mmol/L (74-109 mg/dL)

Urine¹¹

1st morning urine 0.3-1.1 mmol/L (6-20 mg/dL)

24-hour urine 0.3-0.96 mmol/L (6-17 mg/dL)
(average of 1350 mL urine/24 h)acc. to Tietz:⁵**Serum, plasma**

Adults 4.11-5.89 mmol/L (74-106 mg/dL)

60-90 years 4.56-6.38 mmol/L (82-115 mg/dL)

> 90 years 4.16-6.72 mmol/L (75-121 mg/dL)

Children 3.33-5.55 mmol/L (60-100 mg/dL)

Neonates (1 day) 2.22-3.33 mmol/L (40-60 mg/dL)

Neonates (> 1 day) 2.78-4.44 mmol/L (50-80 mg/dL)

Urine

24-hour urine < 2.78 mmol/24 h (< 0.5 g/24 h)

Random urine 0.06-0.83 mmol/L (1-15 mg/dL)

CSF

Children 3.33-4.44 mmol/L (60-80 mg/dL)

Adults 2.22-3.89 mmol/L (40-70 mg/dL)

CSF glucose values should be approximately 60 % of the plasma values and must always be compared with concurrently measured plasma values for adequate clinical interpretation.

Roche has not evaluated reference ranges in a pediatric population. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. *Serum/plasma*: Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days);

urine/CSF: Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Serum/plasma

Repeatability*	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	5.49 (98.9)	0.05 (0.9)	1.0
Precipath U	13.6 (245)	0.1 (2)	0.9
Human serum 1	7.74 (139)	0.05 (1)	0.7
Human serum 2	5.41 (97.5)	0.04 (0.7)	0.7

Intermediate precision**	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	5.38 (96.9)	0.07 (1.3)	1.3
Precipath U	13.4 (241)	0.2 (2)	1.1
Human serum 3	7.61 (137)	0.09 (2)	1.2
Human serum 4	5.28 (95.1)	0.06 (1.1)	1.1

Urine

Repeatability*	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Control level 1	1.54 (27.8)	0.02 (0.4)	1.1
Control level 2	15.7 (283)	0.1 (2)	0.9
Human urine 1	5.00 (90.1)	0.05 (0.9)	1.0
Human urine 2	10.5 (189)	0.1 (2)	1.1

Intermediate precision**	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Control level 1	1.51 (27.2)	0.01 (0.2)	1.0
Control level 2	15.4 (278)	0.1 (2)	0.8
Human urine 3	4.86 (87.6)	0.05 (0.9)	1.0
Human urine 4	10.3 (186)	0.1 (2)	0.8

CSF

Repeatability *	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	5.43 (97.8)	0.04 (0.7)	0.8
Precipath U	13.6 (245)	0.1 (2)	0.8
Human CSF 1	3.04 (54.8)	0.03 (0.5)	0.9
Human CSF 2	8.43 (152)	0.08 (1)	1.0

Intermediate precision**	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	5.37 (96.8)	0.07 (1.3)	1.3
Precipath U	13.4 (241)	0.2 (4)	1.1
Human CSF 3	3.00 (54.1)	0.04 (0.7)	1.5
Human CSF 4	8.30 (150)	0.10 (2)	1.2

* repeatability = within-run precision

** intermediate precision = total precision / between run precision / between day precision

Method comparison

Glucose values for human serum, plasma, urine and CSF samples obtained on a Roche/Hitachi **cobas c 501** analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi MODULAR P analyzer (x).

Serum/plasma

Sample size (n) = 75

Passing/Bablok ¹²	Linear regression
y = 1.000x + 0.118 mmol/L	y = 0.996x + 0.179 mmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 1.64 and 34.1 mmol/L (28.8 and 614 mg/dL).

Urine

Sample size (n) = 75

Passing/Bablok ¹²	Linear regression
y = 1.000x + 0.060 mmol/L	y = 1.001x + 0.045 mmol/L
τ = 0.972	r = 1.000

The sample concentrations were between 0.16 and 39.5 mmol/L (2.88 and 712 mg/dL).

CSF

Sample size (n) = 75

Passing/Bablok ¹²	Linear regression
y = 1.000x - 0.020 mmol/L	y = 1.001x - 0.038 mmol/L
τ = 0.980	r = 1.000

The sample concentrations were between 0.92 and 38.0 mmol/L (16.6 and 685 mg/dL).

GLUC3

Glucose HK

cobas®

References

1. Sacks DB. Carbohydrates. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 4th ed. Philadelphia: WB Saunders 1996:351-374.
2. Krudson PE, Weinstock RS. Carbohydrates. In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 20th ed. Philadelphia: WB Saunders 2001:211-223.
3. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1999:750-785.
4. Kunst A, Draeger B, Ziegenhorn J. In: Bergmeyer. Methods of Enzymatic Analysis, 3rd ed. Volume VI, Metabolites 1: Carbohydrates. 1984:163-172.
5. Tietz NW, ed. Clinical Guide to Laboratory Tests, 4th ed. Philadelphia. WB Saunders Company, 2006:444-451.
6. Tietz Fundamentals of Clinical Chemistry, 6th ed., Saunders Elsevier 2008, 389.
7. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
8. Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
9. Sonntag O, Scholer A. Drug interference in clinical chemistry: Recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001; 38:376-385.
10. Thomas L. Blutglucose. In: Thomas L, ed. Labor und Diagnose, 6th ed. Frankfurt/Main: TH-Books, 2005:193-199.
11. Krieg M et al. Vergleichende quantitative Analytik klinisch-chemischer Kenngrößen im 24-Stunden-Urin und Morgenurin. J Clin Chem Clin Biochem 1986;24:863-869.
12. Passing H, Bablok W, Bender R, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;Nov:26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS C, MODULAR, PRECINORM and PRECIPATH are trademarks of Roche.
Other brand or product names are trademarks of their respective holders.
Significant additions or changes are indicated by a change bar in the margin.
© 2012, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

