Glycaemic Response and Satiety

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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: New Zealand has one of the highest rates of obesity and obesity-related diseases in the world. Claims have suggested that a low glycaemic index diet will curb cravings to overeat by keeping you feeling fuller for longer. However, there is conflicting data regarding the relationship between GI and satiety.

Objective: To determine the effect on measures of satiety and blood glucose of two test foods with different glycaemic indices but otherwise identical composition within a healthy New Zealand adult population.

Design: Randomised double-blinded crossover control trial. The main contribution of this study is to present novel findings in relation to the ongoing scientific glycaemic index debate through the consistency of altering one variable only in humans over a two day trial.

Methods: Seventy-seven participants were randomised to receive the isomaltulose- and the sucrose-sweetened trifles over two testing days, with two to three weeks in between. Only the glycaemic index differed between trifles so that any differences could only be attributed to the rate of digestion of the sugars. Blood samples were collected at baseline, 60 and 120 minutes and were analysed for blood glucose. Subjective satiety was measured using a four-question visual analogue scale questionnaire at baseline, 30, 60, 90, 120 and 150 minutes. Weighed diet records were used to obtain subsequent energy intake for comparison between the trifles.

Results: Blood glucose rose at 60 minutes then declined at 120 minutes, though remained above baseline for both trifles. A statistically significant difference between trifles was observed at 60 minutes following consumption, with a difference of 0.69 mmol/L (95%CI: -

1.12, -0.25; $p<0.05$). Mean satiety AUC did not significantly differ between the isomaltulose- and sucrose-sweetened trifles. There was also no significant difference in subsequent energy intake between the trifles, though there was a tendency for increased energy intake following the consumption of the isomaltulose-sweetened trifle (364kJ, 95%CI: -110, 838; $p=0.133$).

Conclusion: No significant differences were found between the trifles for satiety or subsequent energy intake. Under our test conditions, the glycaemic response was not related to satiety or subsequent energy intake.

Keywords: sucrose, isomaltulose, satiety, glycaemic index, adult, glucostatic theory.

Preface

Fiona Kendall (candidate) conducted this study under the supervision of Dr. Bernard Venn as part of completing a Master of Dietetics. Dr. Bernard Venn was responsible for instigating the research topic, gaining ethical approval, registering this study, manifesting the study design and supervising the write-up of the thesis.

The candidate was responsible for:

- Conducting a literature review on the topic of the glycaemic index and satiety of isomaltulose compared to sucrose;
- Input into study design, including the test food;
- Pre-study engagement with facilitators including verbal and online communication;
- Liaising with study stakeholders over the formation and collation of this thesis;
- Being an involved member of the study team which included designing and printing testing day sheets, setting up and coordinating the sessions; and weighing and producing the test food;
- Data collection, collation and entry of the data;
- Measuring >1500 visual analogue scales for data collation in Microsoft Excel
- Collating and interpreting the weighed diet record results with the support of Liz Fleming;
- Writing and compiling this thesis.

Acknowledgements

To my supervisor **Dr. Bernard Venn**, thank you for your constant support and guidance throughout this process.

To my research partner **Olivia Marchand**, I cannot think of a better person to have been with in the metabolic kitchen for countless hours.

To **Dr. Jill Haszard**, thank you for your expertise and explanations of the statistical analysis.

To **Liz Fleming**, your assistance with Kai-culator at such short notice was much appreciated.

To **Ash** and **Ivy**, thank you for your time and effort with all the blood samples. Data analysis would not have been possible without you.

To **Kieran** and **Ken**, thank you for making data collection run so smoothly.

To my family: **Mum, Dad and Alex**, thank you for everything. I could not have done these past 4½ years without you.

To my **MDiet colleagues**, I have thoroughly enjoyed the chats, discussions and thesis questions in the office.

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List of Abbreviations

GI	Glycaemic Index
T2DM	Type 2 Diabetes Mellitus
MDiet	Master of Dietetics
iAUC	Incremental area under the curve
CHO	Carbohydrate
VAS	Visual analogue scale
BMI	Body Mass Index
kg	Kilograms
m	Metre
µl	Microlitre
EDTA	Ethylenediaminetetraacetic acid
mm	Millimetre
ºC	Degrees Celsius
USA	United States of America
CV	Coefficients of variation
SD	Standard deviation
kJ	Kilojoules
CI	Confidence Interval
AUC	Area under the curve
<	Less than
kg/m^2	kilograms/ metres squared
kJ	Kilojoules

g	Gram
GL	Glycaemic load

1. Introduction

Suggestions have been made that foods with a low glycaemic index (GI) keep you feeling fuller for longer and prevent weight gain by suppressing tendencies to overeat (1). GI is defined as a measure of the glycaemic response exhibited by carbohydrate-containing foods (2). It has been indicated that low GI foods are absorbed more slowly into the bloodstream and have a better glycaemic response compared to high GI foods (3). As a result, the consumption of low GI dietary carbohydrate has been associated with a reduced relative risk (4) and improved control (5) of type two diabetes mellitus (T2DM); and a reduction in the risk of cardiovascular disease (6).

In a country where over 32% of adults are currently obese (7) (with a body mass index (BMI) of >30kg/m2 (7)), New Zealand's growing waistlines have resulted in what has been described as an obesity epidemic (7). Furthermore, excess weight is known to be a key modifiable risk factor for several non-communicable diseases such as T2DM and multiple cancers (7). Unfortunately, health professionals have not yet reached a unanimous decision as how best to address this growing issue. However, advocates for a low GI diet argue that choosing low GI carbohydrates will ensure weight loss (5) and the maintenance of a stable weight (8); and should therefore be incorporated into widespread public health messages (9).

In order to test the effects of foods with different glycaemic indices on satiety and subsequent energy intake, the current study will use isomaltulose as a low GI sucrose alternative to generate a difference in glycaemic response between test foods. Isomaltulose

is a sugar which is found in trace amounts naturally in honey and cane juice (10), and is added to processed foods in larger quantities (11). The disaccharide, also known by its trade name Palatinose™ (12), has a lower GI than sucrose (32 (12) versus 72 (13), respectively). Isomaltulose has been used in Japan since 1985 in products such as confectionary, chewing gum and yoghurt (14) as a non-cariogenic, low-glycaemic sugar (15). A number of toxicology animal studies ((15), (16)) have been carried out. These determined a safe level of consumption so that further human trials could be undertaken in order to measure other end-points such as plasma glucose and insulin levels. Many of these human studies, however, utilised isomaltulose in a single-dose solution (17); and a small sample size ((18), (19)). Isomaltulose has since been approved by Food Standards Australia New Zealand in 2007 for use in New Zealand as a low GI sucrose alternative in products such as breakfast cereals and soft drinks (11). Both sucrose and isomaltulose contain glucose and fructose, though this does not explain sucrose's different rate of digestion of its monomers. This is important because GI studies are often confounded by comparison foods having different composition of protein, fat and fibre, factors that may independently affect the relationship between the food and satiety. This confounding may in part explain conflicting data (20) regarding the relationship between GI and satiety ((21), (22)). Therefore, the main contribution of this study is to present novel findings in relation to GI and satiety when all food factors, apart from the rate of digestion of the sugar, have been controlled for. Hence, the purpose of the current study is to determine the effect on measures of satiety and blood glucose of two test foods with different glycaemic indices but otherwise identical composition within a healthy New Zealand adult population.

2. Literature Review

2.1 Methodology of this Literature Review

The objective of this literature review is to determine whether a low glycaemic index food keeps you fuller for longer.

This literature review aims to:

1. Provide an overview of isomaltulose, isomaltulose biochemistry, isomaltulose metabolism and current uses of isomaltulose;
2. Discuss the current literature regarding isomaltulose, glycaemic index and satiety;

Literature was obtained from Medline via Ovid. Keywords included were ‘isomaltulose’, ‘glycaemic index’, ‘adult’, ‘sucrose’, ‘glucostatic theory’, ‘satiety’ and ‘dietary sucrose’. Further literature was obtained from the reference lists of the published articles that had been previously identified.

2.2 Rationale for research

Carbohydrate-rich foods contribute to a significant proportion of daily energy intake and are a staple part of the diet in many cultures, both in the developing and Western world. Targeting those individuals who want to lose weight or manage their non-communicable disease, claims have been made to promote certain carbohydrates to improve their health outcomes, namely those carbohydrates with a low GI (1). However, the validity of these claims are becoming increasingly important as rates of obesity in New Zealand have increased over recent years (7).

Despite the considerable literature surrounding the effect of GI on satiety, a unanimous decision has not yet been reached as to whether low GI foods do in fact live up to their supposed health benefits. Possible reasoning for this is due to the presence of confounding variables. Therefore, the current study is novel in that only the GI will differ between trifles so that any differences can only be attributed to the rate of digestion of the sugars.

2.3 Isomaltulose

2.3.1 What is Isomaltulose?

Isomaltulose (Palatinose™) is a disaccharide carbohydrate made up of glucose and fructose monomers (**Figure 2.1**) (1). Despite its similarities in taste and appearance to sucrose (2), isomaltulose is only half as sweet (1) as sucrose (table sugar) and contains alpha-1,6 bonds, compared to the alpha-1,2 bonds of sucrose (3).

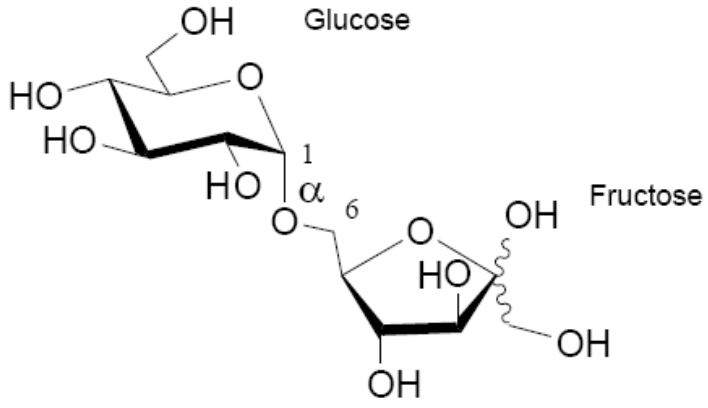

Figure 2.1 Chemical structure of isomaltulose (1)

Isomaltulose is found in trace amounts in honey (1) and sugarcane juice (1). More significantly though, isomaltulose is manufactured using sucrose isomerase (**Figure 2.2**) which converts the alpha-1,2 bond of sucrose into an alpha-1,6 bond (1, 3). This mechanism efficiently produces isomaltulose as the main product of the reaction (4). Commercial production of isomaltulose has resulted in its use as a sugar in Japan since 1985 (2). Isomaltulose has been regarded as safe in the United States of America and has been approved in the European Union (5), but is not yet readily available in New Zealand. Although there are many sucrose alternatives currently available on the New Zealand market, most are sweeteners rather than table sugar replicas. Therefore, there is a gap in the market for this type of sugar substitute.

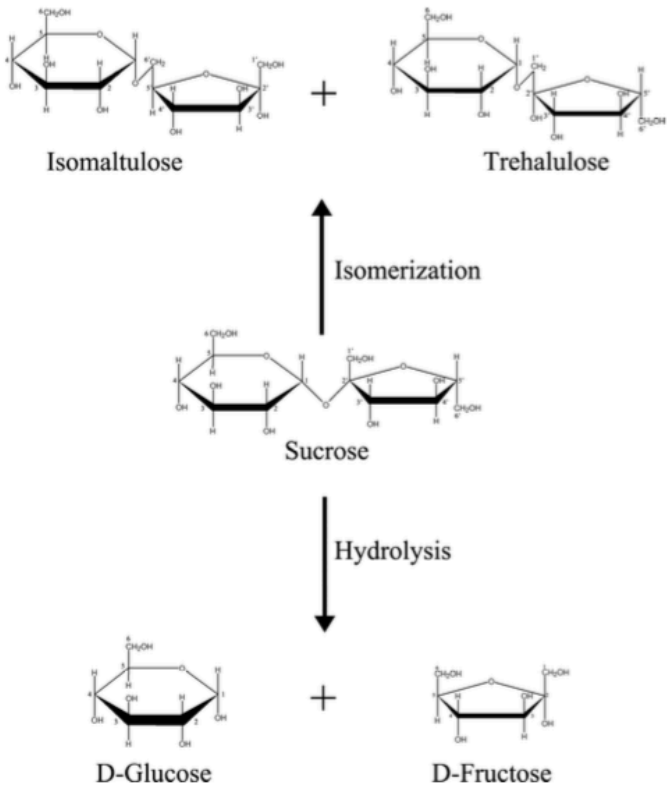

Figure 2.2 Sucrose isomerisation and hydrolysis catalysed by sucrose isomerase (2)

Isomaltulose has a lower GI (explained in **Section 2.5.1**) (6) than sucrose, though it is still completely digested and absorbed in the small intestine (5). Holub et al. (5) was the first study to examine the digestibility and absorption of isomaltulose *in vivo* in humans, as there was little human research prior to 1985 (7). Isomaltulose has been extensively studied in rats, dogs and pigs (2). Noteworthy conclusions from these studies include the discovery that pregnant and non-pregnant rats can tolerate isomaltulose in up to 10% of total daily energy intake for 13 successive weeks with no adverse symptoms ((8), (9)). More recently though, the ingestion of isomaltulose was found to result in significantly less hyperlipidaemia, hyperglycaemia, visceral fat mass, pancreatic islet hypertrophy and adipocyte cell size in rats, compared to sucrose (10). Through natural temporal progression, isomaltulose animal studies have now been superseded by human trials after being deemed safe for human consumption at normal doses (2). Subsequent trials have used isomaltulose for the purpose of evaluating the metabolic effects of the sucrose alternative in humans ((5),(11)). Compared to sucrose, isomaltulose is more appealing to food marketers (1) as a low GI, non-cariogenic (1) sugar. Dental caries place a significant burden on the modern healthcare system (ref). Compared to sucrose, isomaltulose is used by oral plaque bacteria to a much lesser extent (8) and it has been shown to decrease counts of caries-inducing Streptococcus mutans in saliva when compared to sucrose (12). Additionally, isomaltulose elicits a slower rate of digestion (13) and a decreased postprandial blood glucose and insulin response (5).

2.4 Digestion of sugars

Dietary sugars are digested and absorbed in the small intestine (29). Monosaccharides, such as glucose and fructose, are directly absorbed into the small intestine. Whereas, a disaccharide's glycosidic bond needs to be hydrolysed first in order to release its monosaccharides as they are too large to be directly absorbed (29).

Intestinal glucosidases cleave both sucrose, containing a 1,2-disaccharide bond, and isomaltulose, containing a 1,6-disaccharide bond, though the latter more slowly (16). The alpha-1,6 bond is present in natural food sources, such as honey, so humans have the mechanism to hydrolyse the bond, albeit at a slower rate than the 1,2 bond (16). Similarly though, isomaltulose and sucrose are both made up of glucose and fructose monomers (11). However, this does not explain the differences found between sucrose and isomaltulose on blood glucose and plasma insulin concentrations (17).

Most of the carbohydrates consumed in the Western world are in the form of sucrose and starch (33). Starch is comprised of 85% amylopectin and 15% amylose (33). Amylose has a straight chain structure due to its alpha-1,4 bonds, whereas amylopectin has both alpha-1,4 and alpha-1,6 bonds in a bridge-like structure (33). As a result, amylopectin's formation is harder to break down into its individual constituents (glucose monomers) during the digestion of starch (33). Starch digestion begins in saliva but is said to predominantly occur in the lumen of the small intestine by pancreatic alpha-amylase (33).

The enzymes required to hydrolyse the end products of pancreatic alpha-amylase digestion are found within the brush border of the small intestinal mucosal cells (34). Pancreatic alpha-amylase has been shown to hydrolyse only the alpha-1,4 linkages, as the alpha-1,6 linkages remain intact (33). However, isomaltase is able to cleave the alpha-1,6-bond within the alpha-limit dextrins. Isomaltase and sucrase are glucogenic intestinal brush border enzymes which cleave isomaltulose and sucrose, respectively (35).

Carbohydrate digestion occurs in two stages: the luminal phase, which involves pancreatic alpha-amylase, and the membrane phase, which involves intestinal disaccharidases (14). Disaccharidases are located on the terminal membrane of the brush border and hydrolyse the end products of pancreatic alpha-amylase digestion (33). This is responsible for the release of fructose and glucose as the constituents of sucrose, by the action of sucrase (14). Carbohydrate digestion subsequently continues at the brush border where carbohydrate specific enzymes (disaccharidases) act on their corresponding disaccharides to cleave them into monosaccharides to be utilised by the body (14). This is evident through the action of the sucrase-isomaltase enzyme on both isomaltulose and sucrose (14).

2.4.1 Digestion and Absorption of Isomaltulose

Holub et al. (5) was one of the first studies to examine the digestibility and absorption of isomaltulose in humans. Like sucrose, isomaltulose is absorbed in the small intestine, though at a slower rate (2). This contact of nutrients with the small intestine is, in turn, responsible for evoking the satiety mechanism that lower GI foods have been suggested to prolong (15). Isomaltulose undergoes slow hydrolysis of its alpha-1,6-glycosidic bond by the sucrase–isomaltase complex, which is located on the brush border membrane of small

intestinal cells (2). Despite its rate of hydrolysis, absorption of the fully digestible carbohydrate is efficient (16).

2.5 Glycaemic Response and Satiety

2.5.1 Glycaemic Index

GI, first proposed by Jenkins et al. (17), measures the effect of carbohydrate-containing foods on blood glucose (17). Additionally, the numerical classification can be statistically defined as the incremental area under the curve (iAUC) for blood glucose (18). Consumers and healthcare professionals alike are becoming more interested (19) in this model as a means to objectively measure dietary carbohydrate intake. GI is gaining a growing profile through its increasing use in marketing claims by the food industry (20). However, associations have been made in favour of ((21) (22), (23), (24), (25), (18), (26)), and in disagreement of ((27), (28), (29)) the proposed health benefits of a low GI diet. Consequently, the GI notion has sparked global debate (30) as to whether it is a viable measure of health. There is little, but conflicting, evidence regarding the importance of the type of carbohydrate on satiety and therefore energy balance (30).

In order to determine the GI of a food, a portion of food containing 50g of carbohydrate is usually given to at least 10 test subjects over a 120-minute time period (20). However, rather than being based on the subject's physiological response to the food, the GI measure is claimed to derive solely from the properties of the food (i.e. the area under the curve (AUC) for the 50g of carbohydrate) (17). This is due to the fact that the core predictor of the food's GI is its carbohydrate digestion factor (31). GI is a relative glycaemic response depending on how blood glucose levels are influenced. Therefore, the AUC of blood

glucose is written as a percentage of the 'standard' test food (24). Although, it is possible that GI is limited in a realistic setting as in a mixed meal, the differences in GI between foods becomes redundant (32).

Carbohydrates with a lower GI have been said to undergo a delayed rate of glucose absorption from the small intestine, triggering a reduction in the postprandial rise of gut hormones and insulin. As a result, the blood glucose response is suppressed and glucose cellular uptake increases (24). A food's glycaemic response is affected by many factors including its monosaccharide composition, and other food components such as fat and protein (33). The rate that a food is digested may be a key factor of its glycaemic response (17). Previous literature (34) suggests that the rate of glucose absorption can affect the feeling of hunger and satiety.

From a literature search, the results from numerous short term studies (i.e. those that reported findings from a single meal or day) ((35), (36), (37), (38), (39), (40), (28)) showed decreased subsequent hunger and/ or increased satiety after the ingestion of low GI foods compared to the ingestion of high GI foods. These results were predominantly obtained from objectively measured energy intake or a subjective visual analogue scale (VAS) questionnaire. However, only one other study (40) measured the GI of the test foods prior to undertaking the experiment.

Interestingly, Holt et al. (28) proved that boiled potatoes, a high GI food, produced a significantly higher satiety index (SI) score than croissants, also a high GI food, by 7 fold. An AUC measure was used to validate each food in relation to white bread. Additionally,

subjects acted as their own control within each food group, which allowed the unique study design to be a prominent strength of the trial. Therefore, the evidence can be considered the best available to date. Furthermore, in accordance with the current study, Holt et al measured subjective satiety using a 100mm VAS questionnaire (28). Findings showed that SI scores had a positive correlation with fibre, protein and water content. As a result, it has been suggested that the rate of digestion and the glycaemic response influence the feeling of fullness (41).

2.5.2 Satiety

Satiety is a short term factor (41) that defines the feeling of fullness after eating (40). Satiety is measured through its ability to suppress hunger for a certain time period following the cessation of consumption until the next meal (42). There are various objective and subjective measures of satiety. Although there is currently no universal measure of satiety (40), the most reliable method is thought to be through analogue rating scales (40). The VAS is a validated, subjective measure of satiety (43). Visual analogue scales are commonly used to evaluate subjective appetite signals and are comprised of lines of varying length containing phrases of extreme opposition at each end (43). Subjects allocate a mark along the continuum to represent the degree to which each phrase aligns with their degree of hunger (43). Satiety can be objectively measured using three biomarkers: decreases in blood glucose within a 5 minute period, leptin changes within a 2-4 day period negative energy balance, and ghrelin concentrations (44).

Satiety differs from satiation. Satiation is responsible for restricting meal size through its ensuing actions that lead to subsequent meal termination (41). Satiety on the other hand, influences meal frequency as it is responsible for the postprandial events that affect the duration between meals (41).

A noteworthy element of the complex mechanism that influences energy intake is insulin, which acts to regulate both long-term food intake and short-term satiety (45). When the brain senses lower insulin levels, the hormone elicits a hunger response in search of an energy source (45).

2.5.3 Glucostatic Theory

The glucostatic theory is a well-known hypothesis in the literature that was initially described by Mayer (34). Mayer proposed that a low blood glucose concentration stimulates hunger and the onset of feeding, whereas a high blood glucose concentration stimulates satiation signals to cease feeding. This theory is derived from the concept that one's appetite is stimulated when glycaemia drops below a "static" level (46). However, Holt et al. (40) have since suggested that the higher the meal's glycaemic response, the lower the subject's satiety, which therefore opposes the theory. Findings from Leathwood et al. (35) supported this postulation. They showed that compared to a meal containing potato purée (a high GI food), the same meal substituted with bean purée (a low GI food) elicited a lower glycaemic response and subjective satiety. Speculations by Leathwood et al. attributed these findings to a combination of factors including gastric sensations and the rate of glucose absorption.

It is likely however, that in the few studies that have investigated the glucostatic theory, the validity of their study designs have to be considered. In an early trial by Bernstein et al. (47), rather than the participants themselves, the interviewers filled-in the appetite questionnaires. Additionally, in contrast to the current study, satiety questionnaires were filled in hourly following breakfast consumption. This was ensued by treatment administration where questionnaires were completed in 20-minute intervals. Blood samples were taken just before the treatment, just before the meal (30 minutes later) and immediately after the test meal. Therefore, there may have been a crossover of satiety responses between the treatment and food intake.

Stunkard (48) pointed out two major flaws in the aforementioned theory: 1) the concept over simplifies the complex processes that are associated with eating; 2) the theory wrongfully assumes that satiety cues are linked to nutrient absorption in the period immediately following food intake. For the level of satiety felt immediately following ingestion, it is not viable that a significant caloric intake has been absorbed during that time. Therefore, the theory exists but is now outdated. The current study will use a larger sample size to test the hypothesis. Bernstein concluded from his findings that arterial, venous and arterio-venous blood glucose differences had no effect on appetite. Thus, the results of the experiment do not support Mayer's glucostatic theory, and therefore, other factors must contribute to feelings of fullness and hunger.

2.6 Glycaemic Response and Satiety

A few intervention studies ((28), (49)) have shown that GI does not appear to significantly affect satiety. Thirty-nine healthy adult subjects showed that there were no significant differences in satiety between groups (low GI and high GI) for hunger, fullness, or desire-to-eat ratings (49). An impressive difference in GI (43.81 ± 0.99 and 105.26 ± 5.74, for high GI and low GI respectively) was elicited between groups. Forty-eight test foods were used that were representative of a typical Western diet. These included spaghetti, pizza, yoghurt and quiche. However, the differing energy and macronutrient content between test foods is likely to have confounded the results. Therefore, further investigations are warranted. Thus, the current study is an essential addition to the literature.

In contrast to the findings by Alfenas et al. and Holt et al., a randomised controlled trial by Anderson et al. (32) showed that compared to a low GI beverage, an isovolumetric high GI beverage suppressed energy intake after 60 minutes. Additionally, blood glucose was measured at irregular intervals following consumption. However, in accordance with the current study, a VAS questionnaire was completed at regular intervals to measure feelings of hunger and satiety. An inverse relationship was observed between blood glucose concentration and food intake at 60 minutes ($P<0.05$); and subjective appetite ($P<0.05$). However, these findings must be approached with caution as subsequent energy intake was obtained through the provision of a single food (pizza) by the study coordinators at participant request only. Furthermore, the sample size was small (n=14) and only included 18-35 year old healthy males.

Due to the inconsistency of the results by Alfenas et al., Holt et al. and Anderson et al., a clear conclusion cannot be drawn from these intervention studies, especially since study sample sizes were small and the study designs had limitations.

As shown in **Table 2.1**, only one other study (40) measured the GI of the test foods prior to undertaking the experiment, as opposed to using pre-set values from external sources. Thus, the validity of the values in association to the test foods can be questioned. Therefore, the strength of the results from the current study have a design advantage in comparison to those findings from other trials. In addition, only one other study compared was randomised (38); or double-blinded (35). Both of these are crucial aspects in producing an unbiased trial as preconceptions based on suggested claims that low GI foods keep you feeling fuller for longer (50) may influence the results. Additionally, a power calculation was not present in any of the seven other trials. This was a key advantage of the current study as it mostly eliminated findings due to chance through the knowledge of the sample size required. This is especially important as the majority of studies conduct their trials knowing what the main outcome is that they would like to achieve. Furthermore, the majority of trials did not utilise either isocaloric or equal macronutrient test foods, thus influencing the results with confounding factors. Therefore, the current trial is novel in that all study design limitations identified in **Table 2.1** have been controlled for. Thus, there is a gap in the literature for this study.

Table 2.1 Comparison of acute intervention glycaemic index and satiety study designs

Design	Power calculation	Double-blinded	Randomised	Measured GI	Measured blood glucose	Stated GI difference between treatments	Isocaloric treatments	Same macronutrient content between treatments	Blood glucose taken at time of satiety measure
Leathwood et al. 1988 (37)	✗	✓*	✗	✗	✓	✗	✗	✗	✓
Holt et al. 1992 (49)	✗	✗	✗	✓	✓	✓	✓	✓	✓
van Amelsvoort et al. 1992 (60)	✗	✗	✗	✗	✓	✗	✓	✓	✓
Holt et al. 1995 Appetite (46)	✗	✗	✗	✗	✓	✗	✗	✗	✓
Holt et al. 1995 Eur J Clin Nutr (26)	✓	✗	✗	✗	✓	✓	✓	✗	✓
Holt et al. 2001 (61)	✗	✗	✗	✗	✓	✗	✗	✗	✓
Ball et al. 2003 (58)	✗	✗	✓	✗	✓	✓	✗	✗	✓
The current study	✓	✓	✓	✓	✓	✓	✓	✓	✓

2.7 Conclusion

Conclusions from this literature review suggest that on its own, GI is not an absolute determinant of feelings of hunger or satiety. Study design limitations and small sample sizes prevent current evidence from drawing clear conclusions about the effects of GI on satiety alone. Therefore, a more robust trial using isocaloric, equal macronutrient test foods is warranted where only the GI is altered in order to determine the effects of GI on satiety.

3. Objective Statement

The aim of this study was to assess the effects of postprandial glycaemia on satiety.

The study objectives are:

- To test the effects of foods with different glycaemic indices on satiety.
- To test the effects of foods with different glycaemic indices on subsequent energy intake.

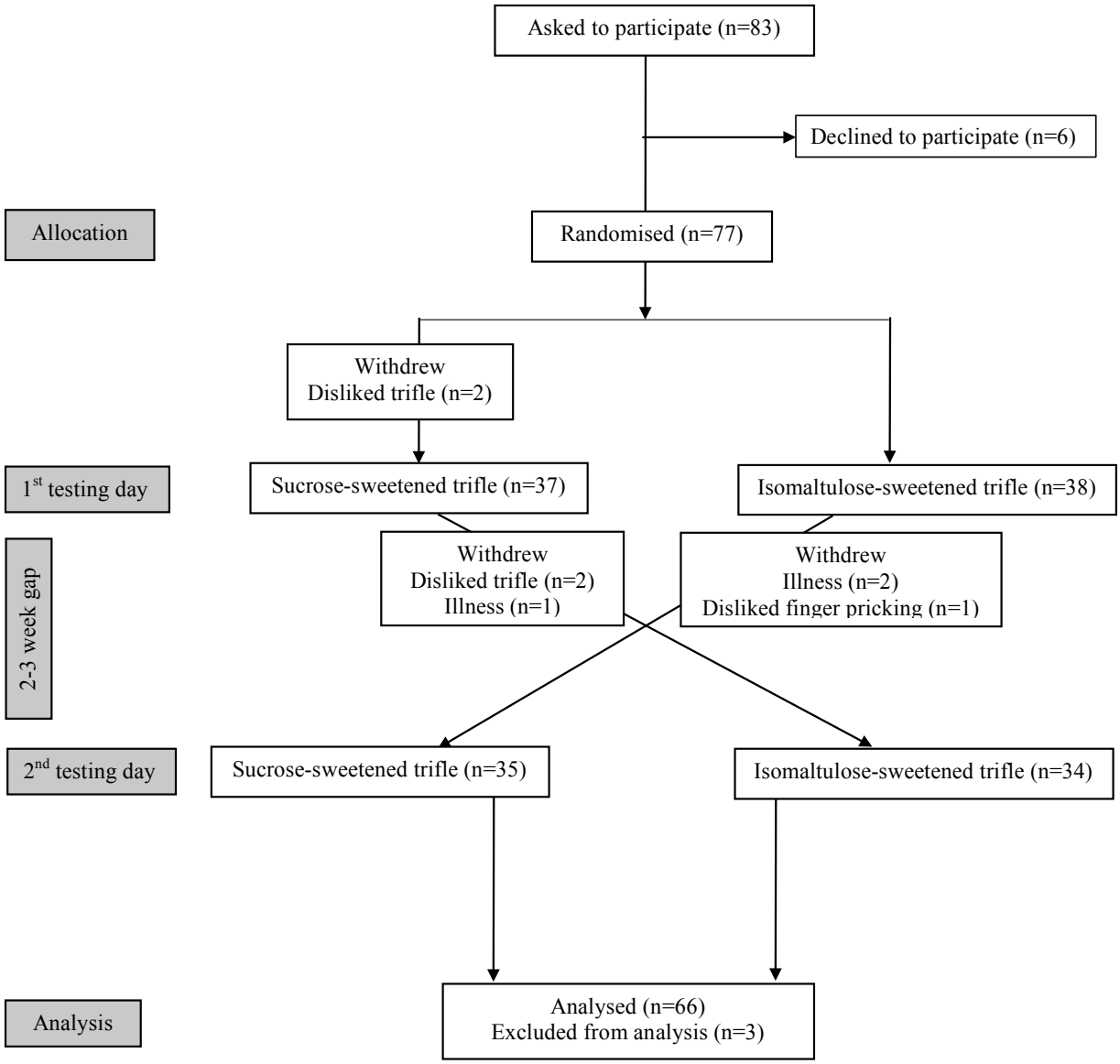

Figure 4.1 CONSORT crossover randomised control trial study design and participant flow diagram

4. Subjects and Methods

4.1 Methods

Data collection took place in the Human Nutrition Undergraduate Laboratories at the University of Otago (Dunedin, New Zealand) between March 3rd and March 31st, 2017. The University of Otago Human Ethics Committee approved this study on 8th February 2017 (number 17/011) (**Appendix A**). The current study undertook Māori consultation through Ngāi Tahu.

4.2 Participants

4.2.1 Recruitment

Participants from this study were a convenience sample of undergraduate human nutrition students from the University of Otago (**Table 5.1**). An information sheet was provided to all participants both in advance of the first testing day and during the session. All subjects gave informed consent before undertaking this study. There were no participant exclusion criteria.

4.3 Randomisation and Allocation

Seventy-seven participants were randomised to receive either the sucrose- or isomaltulose-sweetened trifle, in a cross-over design. Thirty-seven participants were randomised to receive the sucrose-sweetened trifle first and 38 participants were randomised to receive the isomaltulose-sweetened trifle first. A possible source of variability between sexes was controlled for using block randomisation to ensure equal sex distribution for the order in

which the trifles were consumed. Stata Statistical Analysis Software (version 13.1, Stata Corporation 2008) was used to randomise participants. A PhD student from the Department of Human Nutrition coded the trifles such that the study facilitators and participants were blinded to trifle type throughout the entirety of data collection.

As males were a minority of the sample population (n=7), block randomisation was used to ensure they were evenly distributed between groups. Participants were also adjusted for the the order in which they received their trifles.

4.4 Study Design

As shown in **Figure 4.1**, the current study was designed as a double-blinded randomised, controlled crossover trial. The relationship between postprandial glycaemia and satiety was assessed, as well as the relationship between postprandial glycaemia and subsequent energy intake. Blood glucose concentrations and subjective satiety scores were measured at baseline and up to 120 minutes following the consumption of the trifles.

Participants acted as their own controls as they received both trifles on separate occasions. Individual trifle portions were served in opaque red cups so that any visual differences between the trifles could not be detected by the participants (the sponge in the isomaltulose-sweetened trifle tended to be a darker shade than the sucrose-sweetened sponge). Therefore, the double blinded study design was maintained throughout data collection. Participants were allocated 20 minutes to consume the trifle. If a participant was unable to eat the full portion in this time, the trifle container was marked with a line to indicate the amount of

trifle remaining. When the participant consumed the second trifle, he or she was asked to eat to the line to ensure the amount of trifle eaten was the same on both days.

Note: As part of this study, another Master of Dietetics (MDiet) student tested memory and general executive function following the consumption of the trifles. However, the results are not included in this thesis.

4.5 Test Food

Trifles were chosen as the test food to optimise the amount of sugar whilst providing a palatable food (**Appendix B**). This in turn was expected to optimise any differences in glycaemic response between the trifles. The remaining ingredients were identical between the trifles, hence, any differences in glycaemic response could only be attributed to the sugars as all other variables were controlled for.

The nutritional compositions of the trifles were obtained through the use of The Concise New Zealand Food Composition Tables[1]. Values are presented in **Table 4.1**. Both trifles contained exactly the same energy and macronutrient content, with the only difference being the type of sugar used. Weights of sponge, jelly and custard were standardised such that the composite trifle weighed 446g (**Appendix C**). Nine special diets were catered for on request by modifying the basic trifle recipe (**Appendix D**). Though specific dietary requirements affected the macronutrient composition and energy content of the trifles, total carbohydrate content remained consistent throughout. Therefore, the primary aim of the current study was to change only the rate of digestion of the sugars.

The sucrose- and isomaltulose-sweetened trifles were analysed by Dr. John Monro at the Food Industry Science Centre in Palmerston North, New Zealand. Trifles containing 50g of available carbohydrate were tested for their intrinsic carbohydrate content. This enabled the two MDiet students to calculate the quantity of jelly, sponge and custard required to generate a standard trifle serving that contained 98.2g of available carbohydrate. The total available carbohydrate per serving of trifle was generated as a result of the intention to generate the largest differential in glycaemic response whilst maintaining a practically manageable portion size. Actually from table 4.1, the avail CHO was 98.2g and 80.5g is sugars. We wanted to optimise the amount of added sugar in each trifle to generate the largest differential in glycaemic response whilst maintaining a practically manageable portion size.

Testing for Glycaemic Index of the trifles was conducted in 12 people by Glycemic Index Otago, an accredited Glycemic Index testing laboratory at the University of Otago. The portion of trifle for GI testing contained 50g available carbohydrate, and the postprandial responses represented by incremental AUC (iAUC) were compared against the average of three tests of a 50g glucose beverage. GI values of 33 and 44 were obtained for the corresponding isomaltulose- and sucrose-sweetened trifle.

Table 4.1 Nutritional composition of the test meal, per serving[1]

Test Food	Energy (kJ)	Protein (g)	Total fat (g)	Available CHO* (g)	Total available sugars (g)	Sucrose (g)	Dietary fibre (g)
Trifle	2601	15.8	18.6	98.2	80.5	73.2	0.6

*CHO = carbohydrate

4.6 Study Procedure

4.6.1 Data Collection

Participants attended two testing days on different Fridays, with either two or three weeks in between. This gap between sessions was for logistical reasons as well as to mitigate any possible recollections of the taste and palatability of the trifles.

4.6.2 Testing Day Procedure

In order to maintain consistency, participants chose a brand and amount of breakfast cereal to eat on the morning of each testing day. This enabled the candidate to control for appetite and energy intake prior to the session. Following ingestion of their chosen breakfast cereal, participants were asked to abstain from consuming extra food or drink prior to the session. This was to ensure that participants were sufficiently hungry upon arrival. However, if additional food and/ or beverages were ingested during this time, participants were asked to consume those same items on their second testing day. Instructions were given for breakfast cereal consumption to occur at the same time on each testing day. All food and beverages consumed throughout the day, including those ingested prior to the session, were recorded

[1] The Concise New Zealand Food Composition Tables, 11th Edition; 2014

on a weighed diet record sheet (**Appendix E**). This ensured that all food and drink consumed on the morning of the participant's first session was also consumed on the morning of their second session. This record was later entered into the online database Kai-culator.

Upon arrival on their first testing day, all participants (n=75) completed a baseline demographic survey and dietary restrictions questionnaire (**Appendix F**). Participants then signed a consent form to participate in the study (**Appendix G**). The testing days ran from 12 noon to 3:40pm. A session facilitator used stop watches in combination with a scheduled timeline in order to maintain timing consistency throughout the sessions. Water was provided during the testing days and participants were encouraged to replicate their intake across both sessions.

Single weight and height measurements were taken for each participant by a trained research assistant. These measurements were used to calculate body mass index (BMI = weight (kg) divided by height squared (m^2)). A calibrated Seca alpha scales (model 770) (Germany) was used to take weight measurements and a calibrated Holtain limited stadiometer (United Kingdom) was used to take height measurements.

4.6.3 Sample Collection

Finger-prick capillary blood samples were taken at baseline and subsequently at 60 and 120 minutes following consumption of the trifle for the purpose of measuring blood glucose concentration.

Prior to each testing day, collection tubes were pre-labelled with the participant's identification number and the time of blood collection. Tubes were also identically labelled for plasma sample collection following centrifugation. Two days before each session, 10µl of potassium EDTA (sourced from the University of Otago), an anti-coagulant, was added into each tube. By removing the lids and placing an A4 piece of paper on top, the collection tubes were left to dry.

A 500µl capillary blood sample was taken at baseline, then again at 60 and 120 minutes following the consumption of the trifles. Blood collection was undertaken at 60 and 120 minutes to coincide with expected differences in glycaemic response. Trained study facilitators followed a standard blood sampling procedure for blood collection. Peripheral blood flow was increased by applying heat to the hands, then fingers were sanitised with alcohol wipes. Fingers were pricked using a BD microtainer® contact-activated 2.0x1.5mm disposable blue lancet. The first drop of blood was not used as it may have been contaminated or diluted by the alcohol wipe. Subsequent whole blood was extracted into Microcentrifuge collection tubes for analysis.

Following blood collection, the sample tubes were inverted gently seven times. This ensured that the blood sample was fully mixed with the EDTA in order to prevent the blood from clotting. The tubes were centrifuged for 10 minutes at 2000 G. This occurred 5-20 minutes after blood collection. Following centrifugation, the plasma was separated from the packed red blood cells into the labelled tube. Yellow-tipped micropipettes were used to complete

this procedure as it was difficult to utilise a plastic pasteur pipette for such a small volume. An average plasma volume of 160µl was obtained from 500µl of blood. Samples were stored in the freezer at -80ºC for one to four weeks.

4.6.4 Plasma Analysis

Plasma glucose concentration was measured using the glucose hexokinase method on a Cobas c311 auto analyser (Roche Diagnostics, Indianapolis, IN, USA) (**Appendix H**). A Roche enzymatic colorimetric kit was used to run the test. Once the samples were defrosted, plasma glucose concentration was determined within 120 minutes.

Two controls, Precinorm U and Precipath U, were used to enable the range of expected concentrations in the samples to be captured so that values could be interpolated. A two-point calibration was conducted after reagent lot change and as required following quality control procedures. These measures ensured quality control throughout testing. Coefficients of variation (CV) were 1.25%, 0.67% and 1.87% for Roche commercial control material one, Roche commercial control material two and pool plasma, respectively. These values were generated by dividing the mean from the standard deviation (SD), and multiplying the product by 100.

4.7 Study Procedure

Participants completed a VAS questionnaire (43) (**Appendix I**) at baseline, 30, 60, 90, 120 and 150 minutes as a subjective measure of satiety. The VAS questionnaire comprised of

four questions which were answered by marking a vertical line on the 100mm continuum. This represented the degree to which the participants associated with the statements at either end. The VAS questions included: How hungry do you feel? How satisfied do you feel? How full do you feel? How much do you think you can eat? On the left-hand side of the VAS, statements from questions one and four indicated extreme fullness, whereas statements from questions two and three indicated extreme hunger.

The VAS questionnaire (**Appendix I**) was shown to participants prior to data collection. This was to familiarise participants with the layout and requirements of the visual analogue scales to enhance the accuracy of the subjective satiety data. Participants completed one VAS questionnaire at each time period on both testing days.

Data entry was only undertaken by the candidate in order to remove between-person variability. Measurements were taken to the nearest millimetre and were rounded down if the mark intersected the scale at 0.5mm values.

4.8 Dietary Analysis

Kai-culator dietary assessment software (Human Nutrition, University of Otago) was used throughout the study. Following the completion of all testing days, participants entered their weighed diet records from both sessions into Kai-culator. This enabled the candidate to gain full access to the participants' records for subsequent analysis. All diets were sorted to identify any outliers in energy intake or missing data. This ensured that human error was

minimised as participants entered their own diets using only an online "Kai-culator how-to" sheet for guidance (**Appendix J**).

The mean and 95% confidence interval (CI) were generated by subtracting subsequent energy intake on day two from day one following the consumption of the trifles.

4.9 Statistics

4.9.1 Statistical Methods

In addition to analysing each of the four satiety questions separately, an overall appetite scale was calculated as the average area under the curve (AUC) of the four questions. In the case that missing data were present for any one of the four questions, when calculating the overall appetite scale the missing data were imputed as the average of the other questions. Cronbach's alphas were calculated for the overall appetite scale at each time point and averaged.

AUC was calculated between baseline and 150 minutes using the 'pkexamine' command in Stata, using cubic splines. If there were missing data at the first or last time point AUC was not calculated.

Differences in appetite question and scale AUC were determined using mixed effects regression analysis with participant identification as a random effect. Estimates were adjusted for the randomised order in which the participants received their trifles and score before they began eating. Analysis was also undertaken for standardised AUC to enhance

interpretation of the results. The same method was used to estimate differences in subsequent energy intake.

A p-value of <0.05 was considered statistically significant. VAS satiety data were analysed using Stata Statistical Analysis Software (version 13.1, Stata Corporation 2008).
The primary outcome of this study was to investigate whether low GI foods keep you feeling fuller for longer (50).

4.9.2 Sample Size Estimation

Mixed regression models were used to determine the differences between trifles with a random effect for participant identification and adjustment for randomised order. A study sample size of 60 was required to detect a difference of 0.5 standard deviations for all outcomes in standardised form. The study had 90% power to the 1% significance level to detect this difference.

5. Results

Participant flow can be seen in **Figure 4.1**. Following randomisation, two participants who were randomised to receive the sucrose-sweetened trifle withdrew from the study due to lack of enjoyment over the trifle. Three participants withdrew from the sucrose-sweetened trifle group after the first session due to lack of enjoyment over the trifle (n=2) and illness (n=1). Additionally, three participants from the isomaltulose-sweetened trifle group withdrew from the study after the first session due to illness (n=2) and dislike of finger-pricking (n=1). Twenty-three participants did not finish the entire trifle across both testing days. Three participants were excluded from AUC analysis due to missing data.

5.1 Group Demographic Comparison

Baseline participant characteristics are presented in **Table 5.1**. Participants were between the ages of 19 and 51 (as of 3rd April, 2017), with 90.9% of those under the age of 24. 68.2% of the study population were within the healthy BMI range (18.5-24.9kg/m^2), with 36.4% being classified as overweight or obese. The majority of the test subjects were female and of New Zealand European descent.

Table 5.1 Baseline demographics and clinical characteristics

Characteristic	(n=66)
Height†	1.7 (0.1)
Weight†	66 (13.2)
BMI†	23.7 (3.6)
Sex (female)*	57 (86.4%)
Age (years)†	
Male	21 (0.8)
Female	22 (6.1)
Baseline blood glucose (mmol/L)†	
Sucrose	5.2 (0.7)
Isomaltulose	5.1 (0.7)
Ethnicity*	
New Zealand European	40 (60.6%)
Asian	16 (24.2%)
Maori	3 (4.5%)
Other	7 (10.6%)

*Results presented as n (%)
†Results presented as mean (SD)

5.2 Blood Glucose

5.2.1 Evaluation of Plasma Blood Glucose Concentrations

The mean (SD) blood glucose concentrations at baseline, 60 and 120 minutes are presented in **Table 5.2**. Blood glucose rose at 60 minutes then declined at 120 minutes, though remained above baseline for both trifles. A statistically significant difference between trifles was only observed at 60 minutes following consumption, with a difference of 0.69 mmol/L ($p<0.05$)

Table 5.2 Mean (standard deviation) blood glucose concentrations (mmol/L)

Time (minutes)	Sucrose	Isomaltulose	Mean (95% CI) difference (Isomaltulose – sucrose)
0[1]	5.2 (0.7)	5.1 (0.7)	-0.11 (-0.28, 0.07)
60	7.3 (1.7)	6.7 (1.1)	-0.69 (-1.12, -0.25)
120	5.9 (0.9)	6.1 (0.9)	0.23 (-0.06, 0.52)

[1] Baseline blood glucose concentration

5.3 Satiety

5.3.1 Area under the curve

AUC was chosen as the best measure of appetite response across the testing time period on both days, spanning baseline (prior to trifle ingestion) to 150 minutes postprandial. This included a total of six VAS questionnaires by each participant on a given testing day.

The higher the AUC, the higher the participant's appetite response to the trifle. AUC for all four questions were obtained, as well as the overall appetite scale, which was a combination of the questions one to four. This additional marker utilised the same 100mm scale as those questions in the VAS questionnaire. The questionnaire presented opposing statements on the left-hand side of the continuum for questions one and four compared to two and three.
In contrast to a similar trial (51), the current study used AUC instead of iAUC to measure satiety. This was because iAUC does not take the degree of immediate satiety into account following the consumption of the trifles. Therefore, any initial differences in satiety between the isomaltulose- and sucrose-sweetened trifle would be overlooked by iAUC. The measure represented each participant's total appetite response to the trifle, rather than simply how their appetite changed after eating it.

As shown in **Table 5.3**, there were no significant differences in mean AUC for each question following consumption of the trifles. Subsequently, as AUC is a difficult measure to interpret, mean standardised differences were calculated to allow for further investigation of the confidence intervals, which also provided further evidence of the validity of the results from the current study. The effects were standardised in terms of standard deviation in order to standardise the mean (mean=0, SD=1). Therefore, this allowed the unit measurement to be standard deviations. Additionally, a meaningful effect was ruled out as the mean difference and 95% CI were all under 0.3 standard deviations. 0.5 standard deviations suggests a moderate effect size, which was therefore not detected in the current study. Therefore, it is extremely unlikely that there was a difference in satiety between the trifles.

Cronbach's alpha, a measure of internal reliability, was calculated at each time point and ranged from 0.86-0.94. Values >0.7 indicate very good reliability (52), therefore the current study can conclude that the aim was measured with very high internal reliability and hence, good justification to combine these questions into an overall scale of appetite.

Adjustment for special diets, such as vegan or a dairy allergy, did not influence the results. Also, a sensitivity analysis was run that included whether the participants finished their trifle on the second testing day (n=3) or not. This did not change the results in any meaningful way.

Table 5.3 Mean AUC between the sucrose- and isomaltulose-sweetened trifle (mm/minute)

VAS question	Sucrose (mean(SD))	Isomaltulose (mean(SD))	Mean difference (mm minutes) (95% CI)	Mean standardised difference (SD) (95%CI)	P-value
How hungry do you feel?	3628(2457)	3697(2454)	37(-616-691)	0.02 (-0.25, 0.28)	0.97
How satisfied do you feel?	4928(2506)	4886(2667)	-97(-717-523)	0.04 (-0.28, 0.20)	0.91
How full do you feel?	4768(2668)	4899(2859)	23(-673-718)	0.01 (-0.24, 0.26)	0.76
How much do you think you can eat?	4718(2777)	4729(2979)	9(-600-617)	0.00 (-0.21, 0.22)	0.95
Overall appetite scale	4493(2393)	4527(2590)	-9(-589-572)	0.00 (-0.24, 0.23)	0.98

5.4 Subsequent Energy Intake

Subsequent energy intake between the trifles was not significantly different. However, there was a tendency for energy intake to be higher after consumption of the isomaltulose-sweetened trifle, when compared to the sucrose-sweetened trifle. Subsequent energy intake was 364kJ (95%CI: -110, 838; p=0.133, n=66) higher after ingestion of the isomaltulose-sweetened trifle. A sensitivity analysis was run that excluded those participants who did not consume the full portion of their trifle. However, this did not affect the results (343kJ, 95% CI -141, 828; p=0.165, n=63).

6. Discussion

The aim of the current study was to determine the effect of test foods having different glycaemic indices on satiety and blood glucose within a healthy New Zealand adult population. This study tested the hypothesis surrounding claims that low GI foods keep you feeling fuller for longer (50).

Blood glucose rose at 60 minutes following the consumption of the isomaltulose- and sucrose-sweetened trifle, then declined at 120 minutes, though remained above baseline for both trifles. A statistically significant difference was not observed between trifles at 60 minutes following consumption. However, the test conditions of the current study made it difficult to generate a maximal difference in glycaemic response between the trifles without overcompensating for feelings of fullness. This is likely to be because the commercial GI testing of the trifles prior to the sessions was conducted under stricter conditions.

Mean satiety AUC did not significantly differ between the isomaltulose- and sucrose-sweetened trifles. Although there was also no significant difference in subsequent energy intake between the trifles, energy intake following the consumption of the isomaltulose-sweetened trifle tended to be higher than that of the sucrose-sweetened trifle.

To date, no other studies have evaluated the relationship between postprandial glycaemia and satiety, whilst using isomaltulose to lower the GI in order to do so. However, this study showed that the overall GI of a meal is affected by its individual components, thus altering the end GI. As shown in **Table 2.1**, the current study is novel in that previous literature have

not controlled for all other factors other than the glycaemic indices between treatments. Thus, the validity of their findings may have been affected.

In this study, to minimise the effect on GI of non-sugar ingredients, the trifles contained as much sugar as possible to maximise the difference in GI between trifles. However, this led to the serving size being too large as 23 participants did not finish the entire trifle across both testing days. This was also in part due to the acute sweetness of the trifles that decreased its overall palatability.

Both trifles were classified by GI testing as low despite the fact that the GI of isomaltulose alone is 32 (low GI) and the GI of sucrose alone is 65 (medium GI). Therefore, regardless of the fact that all food factors apart from the rate of digestion of the sugar were controlled for, the co-ingestion of fat (53) and protein (25) led to the convergence of the trifle glycaemic indices. Nevertheless, a GI differential of 11 was obtained in the current study. This is approximately the population difference found in dietary GI between the lowest (63) and highest (78) quintile (54). In practical terms, a GI difference of 11 between trifles is realistically achievable in the home setting. This study's GI differential value is likely to have impacted the results. It may have prevented a further increase in the tendency of subsequent energy intake to be higher following the consumption of the isomaltulose-sweetened trifle, compared to that of the sucrose-sweetened trifle. Hence, a significant difference in subsequent energy intake may have been seen between trifles.

This study was an acute trial where participants attended two testing days, with two to three weeks washout. This differed from other studies with similar findings. Two longer-term studies were conducted over eight days (49) and two consecutive 12-week periods (55). It was found in the eight-day trial that there were no significant differences in satiety, food intake or glycaemic and insulinaemic responses between diets consisting of either high and low GI foods (49). The 24-week trial also found no differences in energy intake or subjective satiety ratings between diets containing either high and low GI carbohydrate-rich foods (55). Therefore, despite the differences in study duration between the current study and other trials with similar findings ((55), (49)), study duration does not appear to influence the results.

Several studies have found a relationship between glycaemic response and satiety ((36), (50), (38), (35)). Claims that low GI foods will keep you feeling fuller for longer have arisen from a four-week randomised, crossover controlled feeding study by Chang et al. (50). It was found that compared to a high glycaemic load (GL) diet, a low GL diet significantly increased overall satiety by 7% ($p=0.03$), and decreased hunger in women ($p<0.01$). Unlike the current study, the use of a diverse sample population enabled many subgroup analyses between BMI, gender, percentage body fat and ethnicity to be conducted. However, no differences were found (50). Therefore, it is unlikely that repeating the current study with a more diverse population would affect the results. However, the design by Chang et al. is confounded by fibre content as it greatly differed between diets being higher on the low GL diet by 27g. Therefore, it is likely that the consumption of considerably more fibre contributed to the increase in satiety on the low GL diet. Discrepancies in fibre may

also have been a factor in a study more than two decades previously (35). Bean flakes were used to determine the effects of slow release carbohydrates on plasma glucose and satiety. Findings showed that compared to a potato meal, the bean meal prolonged satiety and decreased the desire to consume a palatable snack. However, the sample size was small (n=6) and the bean meal contained substantially more fibre than the potato meal. From this, it is evident that the strengths of the current study become apparent when the study designs from previous trials are considered. The current study was novel in the fact that the energy and macronutrient content did not differ between trifles. This meant than any differences between trifles could be clearly attributable to GI.

A review outlined plausible reasons as to why there is no consensus regarding the relationship between glycaemic response and satiety (56). It was suggested that within the contradictory literature, a food's GI is often falsely attributed to feelings of fullness. As shown in **Table 2.1**, there are a lack of studies that measure blood glucose, energy intake and appetite simultaneously over an extended period of time. These ideas seem feasible in the ongoing debate regarding the inconsistencies in the relationship between GI and satiety. The review proposed that in the short-term, high GI carbohydrates are better for satiety; whereas over longer periods of time, low GI carbohydrates are better for satiety (56). This is aligned with the glucostatic theory (explained in **1.5.3**) as it was suggested that the impact of GI on satiety is influenced by factors other than their effect on blood glucose.

It is difficult to deduce viable reasons for the difference in findings between the current study and a number of other conflicting studies ((38), (50), (35)). Many variables have been

identified from these studies that are likely to confound the results. One review (57) suggested that prior to food arriving in the gut, the cognitive and sensory signals associated with food have an impact on satiety.

6.1 Strengths and Limitations

The current study used a convenience sample of healthy, young adults, who were mostly female (86.8%). Thus, this limits the applicability of the findings to the wider population. However, the use of a crossover study design strengthened this trial as participants acted as their own controls. This enabled the entirety of the sample population (n=66) to be included in the intervention analysis.

A fundamental strength of this study was that blood glucose samples were taken at the time of completing the VAS questionnaire. This enabled subjective satiety at each time point to be validated by an objective blood glucose measure. Additionally, the GI values from this study were obtained from the trifles themselves, rather than from external sources.

A limitation for this study was that participants generally utilised the full 20 minute time period to consume their trifle. Therefore, though tested in 12 participants, GI testing was able to elicit a truer value for the glycaemic responses produced by the trifles.

6.2 Conclusion

The current study was the first of its calibre to assess the effects of postprandial glycaemia on satiety in healthy young adults. Findings showed no significant differences in mean satiety AUC or subsequent energy intake between the trifles. Blood glucose rose at 60

minutes following consumption and subsequently declined to above baseline levels at 120 minutes, resulting in a statistically significant difference at 60 minutes.

Under these study conditions, satiety was independent of the glycaemic response. Thus, caution should be taken when considering claims surrounding satiety and postprandial glycaemia. Despite the rapidly increasing prevalence of obesity and non-communicable diseases in New Zealand (58), selecting foods based solely on glycaemic response may not be an effective solution.

7. Application to Practice

The practical translation of the GI concept is to swap high GI carbohydrates for low or moderate GI counterparts (59). Following this advice, the main carbohydrate ingredient in trifle (sucrose; medium-high GI) was exchanged for isomaltulose (low GI) and the effect on satiety between trifles containing one or other of these sugars was assessed. Despite a difference in glycaemic response at 60 minutes following consumption, there was no difference in subjective ratings of satiety or objective estimates of subsequent food intake between trifles. A key point to take into practice is that the overall GI of a meal or diet is likely to be affected by the individual glycaemic indices of the constituent foods or ingredients. This is the reason why GI studies are often confounded by comparison foods having different composition of protein, fat and fibre, factors that may independently affect the relationship between the food and satiety. Hence why it was difficult to compare the effects of low GI versus high GI on satiety using trifles that contained exactly the same

energy and macronutrient content. Under our test conditions, the glycaemic response was not related to satiety or subsequent energy intake.

It is important for dietitians to emphasise that consumption of carbohydrate-rich foods should not be based solely on GI. Instead, a focus should be placed on low energy density, satiating foods. This recommendation aligns with the Ministry of Health's Eating and Activity Guidelines for New Zealand Adults (60) which emphasises those carbohydrates that are mostly wholegrain and inherently high in fibre.

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8. Appendices

Appendix A: Ethical approval

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Appendix A: Ethical approval

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.farrondediaz@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

Serves 20

Sponge

- 10 eggs
- 690g castor sugar
- 325g plain white flour
- 125g cornflour
- 10g baking powder

Jelly

- 2200g water
- 625g castor sugar
- 625g lemon juice
- 75g gelatin
- 450g water, for the 2nd measure of water

Custard

- 2850g full fat milk
- 275g cream
- 20 egg yolks
- 150g castor sugar
- 30g cornflour

1. Preheat the oven to 190°C fan bake.
2. Cream the eggs and sugar together for 1 minute.
3. Fold the flours and baking powder into the egg mixture and bake for about 20 minutes, or until golden and cooked through.
4. For the jelly, bring the water and sugar to the boil in a saucepan and boil for 2 minutes. Meanwhile, whisk the gelatine and a cup of lemon juice together in a large jug.
5. Whisk the gelatine mixture into the sugar syrup and stir well. Add the extra measure of water and stir to combine.
6. Strain into serving bowl or mixing bowl and refrigerate until set.
7. For the custard, gradually bring the milk and cream to a simmer over a low heat.
8. In a mixing bowl, Whisk the yolks, sugar and cornflour together.
9. Pour the hot milk and cream over the egg yolk mixture, constantly whisking until it's fully combined.
10. Return into a large saucepan and place back over the heat. Stir gently until thickened.

Appendix C: Preparation methods for special diets

Vegan (n=6): An equi-carbohydrate portion of vegan jelly was prepared using the same cooking method as the basic trifle recipe. An animal-free gelatine alternative (Jel-it-in Vegetarian Gelling Powder, Queen; Australia) was utilised as a direct substitute for the regular gelatine.

Dairy-free (n=2): the basic trifle recipe was used in the absence of the custard component. An equi-carbohydrate portion of the remaining jelly and sponge components were provided.

Gluten- and dairy-free (n=1): the basic trifle recipe was used in the absence of the custard and sponge components. An equi-carbohydrate portion of the remaining jelly component was utilised.

Appendix D: Total trifle weight composition

Total Trifle Weight (per serving) (g)	
Sponge	72
Jelly	194
Custard	180
Total	**446**

Appendix E: Weighed diet record template

Weighed Food Diary						
Student ID:						
	Time	**Amount**	**Food**	**Time**	**Amount**	**Drink**
Breakfast						
Snacks						
Lunch						
Snacks						
Dinner						
Snacks						

Appendix F: Demographic survey and dietary restrictions questionnaire

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 G: Study 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:

Appendix H: Cobas c 311 blood glucose analysis procedure

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Glucose HK

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• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems	
Glucose HK			**cobas c** 311	**cobas c** 501/502
800 tests	Cat. No. **04404483** 190	System-ID 07 6831 6	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
PreciControl ClinChem Multi 1 (20 x 5 mL)	Cat. No. **05117003** 190	Code 391		
PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Cat. No. **05947626** 160	Code 391		
PreciControl ClinChem Multi 2 (20 x 5 mL)	Cat. No. **05117216** 190	Code 392		
PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Cat. No. **05947774** 160	Code 392		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

English

System information
For **cobas c** 311/501 analyzers:
GLUC3: ACN 717
SGLU3: ACN 668 (STAT, reaction time: 7)
For **cobas c** 502 analyzer:
GLUC3: ACN 8717
SGLU3: ACN 8668 (STAT, reaction time: 7)

Intended use
In vitro test for the quantitative determination of glucose in human serum, plasma, urine and CSF on Roche/Hitachi **cobas c** systems.

Summary[1,2,3]
Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.

The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure and liver disease.

Hypoglycemia is less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism or insulin induced hypoglycemia. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glycosuria, to detect renal tubular defects, and in the management of diabetes mellitus. Glucose measurement in cerebrospinal fluid is used for evaluation of meningitis, neoplastic involvement of meninges and other neurological disorders.

Test principle
UV test
Enzymatic reference method with hexokinase[4,5]

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Reagents - working solutions

R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative

R2 HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (E. coli): ≥ 300 µkat/L; preservative

R1 is in position B and R2 is in position C.

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.

Storage and stability

GLUC3	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Plasma: Li-heparin, K_2-EDTA, NaF/Na_2EDTA, KF/Na_2EDTA, NaF/K-Oxalate.

Collect blood by venipuncture from fasting individuals using an evacuated tube system. The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative (NaF) should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~ 7 % in 1 hour (0.28 to 0.56 mmol/L or 5 to 10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.[1]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect

GLUC3

Glucose HK

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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_2O)	
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_2O)	
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_2O 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 triglyceride 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.

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Glucose HK

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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

Plasma[10]		
Fasting	4.11-6.05 mmol/L	(74-109 mg/dL)
Urine[11]		
1st morning urine	0.3-1.1 mmol/L	(5-20 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 (29.8 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®

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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.

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Appendix I: Visual analogue scale questionnaire

HUNT311 Satiety Questionnaire

6

Lab ID:……..

Date: 31/03/17

Time: 150 minutes

Trifle label (circle one) red green

How hungry do you feel?

I am not hungry at all _____ I have never been more hungry

How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Appendix J: Kai-culator how-to

How to use 'Kai-culator'- instructions HUNT311, 2017

Logging on:

Open the Firefox or Chrome web browser

Type https://sybil.otago.ac.nz/dietary/opening.das? into the address line

The 'username' is your university user name and the 'password' is your student ID number

There are clickable 'Help ' and 'FAQ' icons at the bottom right of most screens which give instructions for each screen. Below is a summary of what you need know.

1. How to enter a diet within a project

Select the project e.g. HUNT311 > select Diets > select Records > select New

2. Set up the record

Record the Record ID number, usually the study participant ID number. Kai-culator produces a default ID RRRXXX but override this with your chosen ID e.g. your name to make up 6 letters with no spaces in between. NB be careful when you record the ID. You cannot change it if you make a mistake. You can e-mail Liz to change an ID for you. Send her the incorrect ID and the new correct ID.

- Select *Day#* and Type in 1 or subsequent day number
- Select date that diet was recorded from drop down menu
- Select Start

1. Record food names - two-step process

- Type the first food item in the *Diary item* field. Keep it broad to start with by recording e.g. 'milk' rather than the specific type/ brand. You don't need to write measurements/ quantities in at this stage.
- To select time click the up/down arrows to find the correct meal time or type the time in 24-hour clock format. It's best if you record the time in at this stage. Click or the 'Return' key between food items. Add more food items. Click the *'Save'* icon.
- Click the 'Continue diet record' icon below the table to go to the next screen.
- Now choose the specific foods from the *Food list items* to match your descriptions.
- Clicking on foods in the *Food item* column triggers an automatic search for matching/close items from the *Food list items* (below *Food diary)* If a match still does not exist see the hints below.
- A food match is selected by double-clicking it. Its description is now entered into the diet.
- Changes are not automatically saved - a green corner in a table cell means unsaved data
- Click the *'Save'* icon (far left icon below the *Food diary*) to commit these changes

Hints: The foods displayed in the *Food list items* can be expanded or contracted by changing the selection criteria in the fields *'Starts with'* and *'contains'*

a) Frequently the first word is a generic name such as 'bread' or 'beef'
b) Leave the *'contains'* field blank to see all the options for foods that start with the selected name in '*Starts with*'. Add a descriptor in the *'contains'* field to shorten the list to foods containing the descriptor, e.g., wholemeal or porterhouse.
c) If *'Starts with'* does not find the food you are looking for choose *'Contains'* from the drop down list and click search.
d) To see all of the foods in a food group click on the drop down box and select the food group name. Scroll up and down to familiarise yourself with foods in the database. (Note: leave the other fields blank)

- Enter the food amounts. Click on *Amount* for each food and select the appropriate amount from the three methods available; grams; measure descriptors; volume/shape. **NB**: mls won't work on the left hand box when typing in quantities. You have to enter liquids on the right hand box and select mls as the unit and then type in e.g. 180 for 180ml.

Hints

a) Choose only one method for recording an amount as Kai-culator has a hierarchy of selection if more than one method is recorded, i.e., a value in the *Measure descriptor 'How many'* column takes priority over a value in the *Volume 'How many' field*. which takes priority over a value in the *Amount* column
b) Click the "Save" icon to commit these changes.
c) If there is no density for a food a volume can not be converted to grams and so the nutrient amounts for the food will not be calculated.

1. Recipes

There are two ways to create new recipes within 'Kai-culator';

- New project *'List'* recipes. This method is useful for recording generic recipes or for participants own recipes where they have **recorded the raw ingredient weights or measures and then cooked the food**.
- Recording a participant's own recipe within their diet record. This method is useful for foods which are not cooked, e.g. **jelly**, a sandwich or an assembly of already cooked ingredients, e.g. cooked pasta, pasta sauce and parmesan cheese topping.

How to create a new 'Project' recipe in the project 'Recipe Database'

- Click on your project e.g. HUNT311 2017
- Click Composition Data>Recipes
- Click the icon
- Type in the **name of your recipe and your initials** e.g. spaghettibolognese FK > click *'OK'*.
- Click the name of the recipe, then click 'Edit recipe' icon - new tables appear on the right of the screen. The top one is the recipe table.
- In the recipe table select the cooking method from the drop down menu.
- Type in the cooking time and temperature (100ºC for boiled, steamed, microwaved; 180ºC for baked, roasted, stir-fried, fried. 220ºC for grilled, barbecued).
- Click 'Save' icon.
- Record the moisture change value - see hints below. Click 'Save' icon.
- Add ingredients - see hints below. Click 'Save' icon.
- Record ingredient amounts - Click the 'Amount' field and record the amount as grams, or a measure descriptor. Click *'OK'*. Click 'Save' icon.
- Record the Retention factor - see hints below. Click 'Save' icon.

Click the 'Calculate recipe' icon and check that the nutrient values in the left hand right table change from '-0.1g' to a positive value. Check the water value is not zero. If it is zero, check you have recorded the moisture change value field (see hints below)

Hint: How to record the moisture change value

Moisture change values are found in two places in Kai-culator…

'Kai-culator' recipes – within a project the ANS0809 recipes can be found in 'Composition Data /Recipe Database' then click the 'Switch view' icon.

- Search for a similar recipe. Type food name in *'Starts with'* field and click *'Search'*.
- Click on an appropriate recipe and click on the *'View recipe'* icon.
- Note the moisture factor.

USDA moisture factors list is found in the main Kai-culator menu

- Click 'Data' > 'Food Composition Databases' > 'Moisture factors'
- Scroll through the long list of foods to find similar recipes and note the average moisture value for that type of recipe.
- Click 'Save' icon.

Hint: How to add ingredients

- Click the icon. The ingredient number appears.
- Click in the *'Food item'* field which will turn pink.
- Type the ingredient name in the *'Starts with'* field and click *'Search'*. Double click on the appropriate ingredient which will appear in the *'Food item'* field.
- **Note:** Make sure you choose raw ingredients for Project Recipes, except where pre-cooked items are included in a recipe. The moisture change and the retention factors will 'cook/convert' raw ingredient nutrient values to cooked values.

Hint: How to record the retention factor

- Click the *'Retention field'* for each ingredient and select the appropriate retention factor for the type of food and cooking method from the drop down menu.
- Click 'Save'.
- Repeat for all ingredients.
- **Note: Some foods do not have retention factors applied e.g. sugars, fats and oils, so click 'Not applicable' factor.**

Participants' own recipes

The participants' own recipes can be entered within the diet for uncooked recipes, e.g. smoothie, salad or sandwich or an assembled cooked food recipes, e.g. pasta and sauce if they have recorded the amounts of raw or cooked ingredients and the proportion of the recipe they consumed. If they don't state the proportion or only state they consumed a weighed or measured amount such as '125g' or '1 cup' it might be easier to record their recipe as a 'List recipe', see above.

How to enter a participant's own recipe within a diet record as cooked ingredients and raw amounts

- Click 'Food item' cell so it turns pink > Click the recipe icon on the right of the same line (A new recipe screen will come up)
- To add new ingredients Click icon
- Type in the name of the first ingredient > Click OK.

- Type in the name of the second ingredient > Click OK repeat for all ingredients.
- Click cancel and return to the Food Diary Reconciliation recipe screen. Click 'Save '
- Click the 'Food item' field for the first ingredient. Check below the table to see if the food item you want is in the 'Food list', remembering to choose the cooked version of the food if the recipe is an assembly of cooked ingredients. Double click on the appropriate Food item.

  Hints: The foods displayed in the *Food list items* can be expanded or contracted by changing the selection criteria in the fields *'Starts with'* and *'contains'*

  e) Frequently the first word is a generic name such as bread or beef
  f) Leaving the *'contains'* field blank gives all the options available for foods that start with the selected name in 'Starts with'. Adding a descriptor in the *'contains'* field shortens the list to foods containing that descriptor, e.g., wholemeal or porterhouse.
  g) If the *'Starts with'* field does not find the food you are looking for choose *'Contains'* from the drop down list and click search.

- Click the 'Amount field'. Enter the amount in the 'g/ml' amount field, the measure description field or shape field.
- Repeat the last two steps for all ingredients
- Click 'Save recipe' [redacted]icon in the bottom left corner of the table
- Click 'Exit' icon (to return to the Food Diary Reconciliation screen
- Click on the 'Amount field' > Type in % the participant consumed> Click OK
- Click 'Save' icon. Click 'Exit' icon