The effect on satiety of ingesting sucrose and isomaltulose sweetened beverages

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Abstract

**Background:** It is a commonly perceived perception that low-glycaemic index (GI) foods keep you feeling fuller for longer due to prolonged postprandial glycaemia (PPG). Thus, it is thought that low-GI foods could promote weight loss. However, the published findings on the relationship between PPG and satiety are inconclusive. Inconsistency in results could be attributable to factors in food other than the food’s glycaemic-inducing properties. For example, foods chosen on the basis of GI may also differ in factors such as palatability, macro- and micronutrient content, fibre and energy.

**Objective:** To determine the effect of PPG on satiety using sucrose (GI= 65) and isomaltulose (Palatinose™) (GI= 32) sweetened beverages. Sucrose and isomaltulose are both disaccharides comprising the monosaccharides glucose and fructose; both sugars are fully digested and absorbed; the difference between them being the glycosidic bond that is digested more rapidly for sucrose than it is for isomaltulose.

**Design:** Double-blinded, randomized controlled crossover trial, in which 77 participants were recruited to measure satiety outcomes, 12 of who volunteered for blood glucose measurements to determine glycaemic response.

**Methods:** Twelve volunteers were recruited for blood glucose measurements at baseline, 30, 60, 90, 120, 150, and 180-minutes after consumption of a sucrose or isomaltulose sweetened beverage, two weeks apart. Blood samples were analysed for blood glucose and insulin concentrations. The full 77 participants were randomized to receive one of each beverage, two weeks apart. Satiety was measured via visual analogue scales (VASs) at the same time points as the blood glucose measurements. VAS questions consisted of; “How hungry do you feel?”; “How satisfied do you feel?”; “How full do you feel?”; And “how much do you think you can eat?”. Weighed diet records were kept from 5:00pm (180-minutes after beverage consumption) until 12:00am, and were used to compare subsequent energy and macronutrient intake.
**Results:** Glycaemic and insulinaemic response, measured by incremental area under the curve (iAUC), differed significantly between the sucrose and isomaltulose beverages. Mean blood glucose concentrations differed by 44mmol/L (95% CI: -70, -18; P= 0.003) and mean blood insulin concentrations differed by 1883µIU/L (95% CI: -2846, 921; P= 0.001). VAS questions, measured by area under the cure (AUC), showed no difference in hunger (P= 0.699), satisfaction (P= 0.924), fullness (P= 0.780) or prospective food intake (P= 0.341), between the test beverages. No significant difference in subsequent energy (95% CI: -845, 267; P= 0.306), fat (95% CI: -13.3, 0.2; P= 0.056), protein (95% CI: -8.8, 4.3; P= 0.498) or carbohydrate intake (95% CI: -17.2, 20.5; P= 0.864) was found between the beverages.

**Conclusion:** There was no difference in measures of satiety following ingestion of sucrose and isomaltulose sweetened beverages despite differences in PPG. These findings indicate that at the differences in glycemic responses attained in this study, satiety is independent of glycaemia per se. Any differences found between foods chosen on the basis of GI could be attributable to food properties other than the glycaemic-inducing potential of the food.

**Key words:** sucrose, isomaltulose, glycaemic response, glycaemic index, satiety.
Preface

The candidate conducted this study under supervision of Dr. Bernard Venn in five-months as part of completing a Master of Dietetics (MDiet). Dr. Bernard Venn was responsible for creating the research topic and study design, gaining ethical approval and supervising the write up of the thesis.

Data were collected on cognition by a fellow MDiet colleague, these results will be presented in a separate thesis.

The candidate was responsible for the following:

- Completing a literature review on the topic of glycaemic response and satiety.
- Development and preparation of the test beverages with MDiet colleague.
- Preparation of the sensory testing, including recruitment of participants with MDiet colleague.
- Conducting the sensory testing with MDiet colleague.
- Development of questionnaires and recording forms used for data collection with MDiet colleague.
- Input into study design: development of laboratory timeline, instructions for participants and organisation of laboratory testing sessions.
- Taking anthropometric measurements of participants.
- Data collection, data cleaning, data entry, tables and figures.
- Measuring 4,984 visual analogue scale (VAS) lines for data collation in Microsoft Excel.
- Collating the weighed diet record data in Microsoft Excel.
- Input into statistical analysis.
- Interpretation of results.
- Writing this thesis.
Acknowledgements

To my supervisor Dr Bernard Venn, your knowledge and feedback has been invaluable in this process. To my co-supervisor Dr Tracy Perry, thank you for your additional support along the way. I’ve appreciated all the time you’ve both put into helping me through this research process.

Ivy, Michelle, Ken and Ash for your organization and ongoing help in the laboratory through the experimental phase of this study. Liz Fleming, for taking your time to explain the ins and outs of Kaiculator to me. Dr Mei Peng for your knowledge and help with conducting the sensory testing.

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My wonderful flat mates for keeping fifth year full of fun. Nick for your constant support, positivity, library company and of course, always keeping me caffeinated! Library dates and study breaks wouldn’t have been as enjoyable without you all.

Finally, to Mum, Dad, Nana and Neil – thank you for all your love, support and encouragement over the last five years. It could not have been done without you all.
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>CK</td>
<td>Celeste Keesing</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>g</td>
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<td>GI</td>
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<td>GL</td>
<td>Glycaemic load</td>
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<tr>
<td>iAUC</td>
<td>Incremental area under the curve</td>
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<td>Kg</td>
<td>Kilograms</td>
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<td>Kj</td>
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<td>MDiet</td>
<td>Master of Dietetics</td>
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<td>µL</td>
<td>Microlitre</td>
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<td>µIU/L</td>
<td>Micro international units per litre</td>
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<td>mL</td>
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<td>mm</td>
<td>Millimetre</td>
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<tr>
<td>mmol/L</td>
<td>Millimol per litre</td>
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<td>n</td>
<td>Number of participants</td>
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<td>PPG</td>
<td>Postprandial glycaemia</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>VAS</td>
<td>Visual analogue scale</td>
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2. Introduction

A major dietary change seen in recent decades is the shift from high quality carbohydrates to fibre depleted processed carbohydrates, coincident with the rising rates of obesity, type 2 diabetes mellitus and coronary heart disease (CHD) (1). The relevance of carbohydrates and postprandial glycaemia (PPG) to metabolic disease risk has been debated since the first glycaemic index (GI) publication in 1981 (1). Although evidence is conflicting, the Glycaemic Index Foundation claims that low-GI keeps you feeling fuller for longer (2). The suggestion that glycaemic responses elicited by carbohydrate containing foods may be causal to the rising rates of metabolic diseases (1), requires attention to be paid to the impact of PPG on satiety.

GI was introduced to determine the effect of different carbohydrate containing foods on PPG (3). Foods with a low-GI are characterised as releasing glucose into the blood at a slow steady rate, whilst foods with a high-GI are characterised as causing a rapid spike in blood glucose and a greater overall glycaemic response (4). The difference in glycaemic response is thought to be attributed to the rate at which carbohydrates are digested and absorbed, with some low-GI foods thought to have the more favourable glycaemic impact on health and disease parameters (5).

Low glycaemic foods have been shown to enhance satiety when compared to high glycaemic foods (4). It was originally thought that blood glucose concentrations regulated food intake, known as the glucostatic theory (6). This theory suggests that increased blood glucose concentrations promote satiety, whilst low blood glucose concentrations lead to increased feelings of hunger (6). However, evidence of a low glycaemic response
correlating to higher satiation is conflicting and more recent evidence suggests blood glucose concentrations are not the primary determinant of satiety (7). The inconsistencies between studies may be explained by factors other than the glycaemic effect such as: cooking and processing methods, the presence of fibre, and the presence of other macronutrients (4). The differences could also be attributed to variable study designs and methods used to measure satiety, such as visual analogue scales (VASs) or weighed diet records (8).

The purpose of this study was to compare the effect of a sucrose (medium-GI) and a sucrose alternative (low-GI) beverage on satiety. The sucrose alternative used was isomaltulose. Although a fully digestible carbohydrate, it is digested at a slower rate than sucrose due to its more stable alpha-1,6 glycosidic bond, compared with sucrose’s alpha-1,4 linkage (9). This data will be novel because all properties of the beverages will be controlled for, leaving only the difference in digestion as an explanatory factor if outcomes differ.

Therefore, this double-blinded, randomised controlled crossover trial was designed to assess the satiating potential of the two sugars, in the absence of confounding factors. The results can be used to provide further evidenced based recommendations on low glycaemic sucrose alternatives, in relation to reducing food intake for weight management, minimizing risk of obesity, diabetes and CHD.


3. Literature Review

3.1 Introduction

The purpose of this literature review was to investigate the effect of postprandial glycaemia (PPG) on satiety.

Specifically, this review focuses on:

- Providing an overview of glycaemic response.
- Defining glycaemic index (GI) and glycaemic load (GL).
- Discussing GI and GL in terms of weight management.
- How glycaemic response may affect satiety, including a description of the glucostatic theory.
- Comparing the properties of isomaltulose to sucrose.
- Recognising gaps in the current literature around measuring the effect of glycaemic response on satiety, in isolation of confounding meal components, to develop a rationale for this study.

3.2 Literature review methodology

Literature was sourced from the following online databases: Medline, Scopus, Science Direct and Pubmed. Search key words included: ‘glycaemic response, ‘glycaemia’, ‘glycaemic index’, ‘glycaemic load’, ‘isomaltulose’, ‘glucostatic theory’, ‘glycaemic response and satiety’, ‘glycaemic index and satiety’, ‘isomaltulose and satiety’. Further references in articles found from these online searches were used. Articles from 1956 to 2017 were included in this literature review.
3.3 Glycaemic response

Glycaemic response or PPG refers to the change in blood glucose levels after consuming a carbohydrate containing meal (10). Glucose concentrations begin to rise approximately ten-minutes after the start of a meal due to the absorption of carbohydrates (10). The rate at which this occurs depends on the amount and type of carbohydrate consumed (11). In a healthy individual, blood glucose levels typically rise no higher than 7.8mmol/L in response to a meal and return to pre-meal concentrations within two to three hours (12).

However, glycaemic response can vary widely between individuals (13), due to a host of factors. It has been found that those from South Asia elicit a glycaemic response that is two to three times larger than Caucasians, indicating considerable metabolic differences between ethnicities (14). It has also been found that differing dentition, chewing rates and eating behaviour between individuals results in different particle sizes which influences the magnitude and pattern of the glycaemic response (15).

Additionally, approximately 150 million people worldwide have Diabetes Mellitus with this number expected to double by 2025 (16), signifying a large proportion of the population with insulin resistance. Insulin resistance results in a higher glycaemic response due to glucose accumulating in the blood, unable to enter body cells (17), providing another mechanism by which glycaemic response could differ between individuals.

3.3.1 Glycaemic index (GI)

The GI concept was introduced in the early 1980’s as a ranking system for carbohydrates based on their blood glucose raising ability (18, 19). GI is defined as the incremental area
under the glycaemic response curve elicited by a test food containing 50g of available carbohydrate, expressed as a percentage of the area under the curve (AUC) elicited by 50g of glucose from a reference food (or mean of two to three glucose references), in the same subject (19). The mean GI value of the set number of participants (usually 10 or 12) is used to determine the overall GI value for that food (20). Carbohydrate based foods have been classified into three categories according to their GI to help predict glycaemic response. Foods with a low-GI are defined as <55, intermediate-GI as 55 – 70 and a high-GI as >70 (21).

### 3.3.2 Glycaemic load (GL)

Both the type and quantity of carbohydrate consumed influence the glycaemic response (3). However, GI only takes into account the type of carbohydrate and is based on a fixed amount of available carbohydrate (11). In order to improve the predictability of glycaemic response, GL was introduced to include the amount of available carbohydrate consumed (GL = GI X available carbohydrate (g) / 100) (4). Thus, GL can assess the total glycaemic effect of the diet (11).

### 3.3.3 Glycaemic response and weight management

Diets that elicit a low glycaemic response have been suggested as a means of improving chronic health conditions such as diabetes mellitus, obesity and risk of CHD (22). However, literature is inconsistent and it appears the health benefits of a low-GI diet are more prominent in overweight and/or insulin resistant individuals, than healthy individuals (1).
From observational data, when ranked into percentiles of GI and GL, it has been found that GI or GL are unrelated to BMI in the populations studied (22-25). Data from the European Prospective Investigation into Cancer and Nutrition showed no interaction with BMI status or other anthropometric measurements in relation to quintiles of GI or GL intake (25). In an observational study conducted in 646 elderly men, those with a higher dietary intake of GI had a lower BMI, although the difference was only minimal with a 1.1kg/m^2 difference between the low and high tertile of energy-adjusted GI intake (26). Thus, data from observational studies do not support the contention that low glycaemic diets are beneficial for weight loss.

In a review that analysed 13 long-term human intervention studies (>6 days) that used low or high glycaemic treatments, a greater weight loss on a low glycaemic diet was seen in two studies, greater weight loss on a high glycaemic diet was seen in one, and no difference was recorded in ten (27). The average weight loss across these 13 studies was 0.2kg on the low glycaemic diet and 0.7kg on the high glycaemic diet (27). Thus, these results provide no evidence that low glycaemic diets promote weight loss any more than high glycaemic diets. The CARMEN multicentre trial that was conducted in 398 moderately obese adults, found that six-months ad libitum intake of low-fat diets rich in either low or high glycaemic carbohydrates, reduced body weight by 1.6-2.4kg compared with the normal-fat control diet (28). Study findings indicate no significant difference between the low and high glycaemic carbohydrate diets. Therefore, in this particular study a reduced fat diet rich in carbohydrate was beneficial for weight loss regardless of the type of carbohydrate.
3.4 Glycaemic response and satiety

Satiation determines meal size as it is the mechanism that leads to meal cessation (21). In contrast, satiety impacts meal frequency by suppressing hunger for a period of time (21). In order to measure these subjective feelings of appetite, visual analogue scales (VASs) are often used (29). VASs are composed of lines with words anchored at each end describing opposing statements, and participants allocate a mark on the continuum to describe their feelings of: hunger, satisfaction, fullness, and prospective food consumption (29). Satiety can also be measured by food intake at the next meal via weighed diet records.

A systematic review found evidence from short-term studies (<1 day) that low glycaemic foods have a higher satiating effect than high glycaemic foods (21). However, confounding may have been an issue because studies for this systematic review were selected for analysis if the energy and macronutrient composition of the test meals were similar. However, this did not guarantee there were no differences in fat and protein content of the test foods, components that could have an effect on satiety independent of GI (21). Furthermore, selection criteria did not include fibre content or palatability of the test foods, which both influence satiety independent of the GI (21). Therefore, whilst 12 out of the 18 short-term studies reviewed showed evidence of an increase in satiety with low glycaemic foods, this connection is actually very weak (21). Due to the increasing presence of confounding factors in longer term studies (>1 day), the systematic review did not make any conclusions on low glycaemic diets and satieogenic health benefits in the long-term (21).
In another review, a subset of studies that investigated GI and satiety short-term (<1 day) whilst controlling for all test meal components, were examined independently of the poorer controlled studies that had differences in test diets such as energy density and palatability (8). These studies found no correlation between GI and satiety using VASs. However, all studies that also used weighed diet records to measure subsequent energy intake found that consumption of high glycaemic foods promoted higher energy intake at the next meal when compared with low glycaemic foods (8). These results convey the importance of using both VASs and weighed diet records to determine satiety.

An acute feeding study fed 1000kJ isoenergetic portions of food to fasting subjects, after which a standard meal was provided and ad libitum food intake was recorded (30). As most of the carbohydrate foods used in the study were less energy dense than the other servings of food, participants had to eat a larger volume of these foods to create isoenergetic portions (30) It was found that food volume and thus energy density was a stronger predictor of individual satiety than the foods glycaemic impact. Furthermore, there was no significant relationship found between satiety and blood glucose concentrations (30), as the glucostatic theory suggests (6).

Fewer long-term studies have been conducted comparing low and high glycaemic diets. However, in many longer term studies the correlation between low glycaemic foods and an enhanced feeling of fullness has not been found. It is important to note that isolating the effect of low glycaemic foods long-term can be difficult due to numerous confounding factors such as: meal duration, macronutrient composition, energy density.
textural properties, fibre content and palatability (30-32).

An 8-day randomized controlled trial found that consumption of only low or high-GI foods (served alone or in mixed meals with identical macronutrient composition), did not elicit a significantly different glycaemic response (31). This may have been attributed to no set meal duration, as slower rates of eating moderates glycaemic and insulinaemic responses (31). Subsequently, there were no significant differences in appetite or energy intake (31). A similar study investigating the effect of a low-fat, high-carbohydrate diet, with either low or high-GI carbohydrates (with all other dietary components being equal) over 10-weeks had comparable results (32). This parallel, randomized controlled trial found no significant difference in energy intake or changes in body weight between the two diets (32). Additionally, diaries filled out on a daily basis by the participants showed no difference in ratings of hunger or fullness (32). These results support the notion that carbohydrate content is a greater determinant of satiety than GI itself (30, 32).

The current available literature is equivocal regarding the predictive power of the GI of foods and subsequent satiation. While there is some evidence from short-term studies that show low glycaemic foods have higher satieogenic properties than high glycaemic-foods, the current long-term studies do not support this hypothesis.

3.4.1 The glucostatic theory

Jean Mayer proposed the glucostatic theory in 1953 (33). The proposition was that low blood glucose concentrations trigger hunger and the onset of feeding, whilst high blood glucose signals satiety and prevents further feeding (34). This short term energy regulation mechanism relies on the concept that changes in blood glucose concentrations
are detected by glucoreceptors, possibly in the hypothalamic centres (6). Lower and slower glucose and insulin responses are believed to promote satiety, whilst large increases in blood glucose and insulin concentrations may result in a hypoglycaemic undershoot, which leads to an increase in hunger (34). However, evidence that blood glucose is the primary determinant of satiety and food intake in humans is inconsistent (7).

A study conducted to determine if changes in hunger were related to changes in blood glucose concentrations, found that hunger increased after transient declines in blood glucose, and no change in hunger occurred when blood glucose concentrations were stable (35). A review of multiple studies suggests that a trend toward hypoglycaemia might induce excess energy intake, weight gain and impaired glucose tolerance, in agreement with the glucostatic theory (36). Thus, a low-GI meal is predicted to result in a more stable blood glucose response, inducing satiety and reducing energy intake.

However, a number of studies have been unable to demonstrate a relationship between blood glucose concentrations and appetite. A study that tested the effect of blood glucose levels on food intake and appetite, conducted food consumption tests three times per week for three weeks on nine healthy adult males (37). These participants were given a fixed breakfast at 7.30am, and at 11.30am the test treatments were administered (different concentrations of intravenous and intragastric glucose) (37). At 12.00pm the subjects were provided a test meal which they were permitted to consume ad libitum and the amounts of all food and macronutrients were measured (37). The results of this study
showed that although the test treatments resulted in three distinct blood glucose concentrations, no significant difference was found in terms of total caloric intake, total macronutrients, or feelings of appetite (37). The use of intravenous and intragastric administration of glucose removes confounding factors, such as palatability, gastrointestinal feelings and the presence of other nutrients, making this a strong study that does not support the glucostatic theory.

There are a number of other possible mechanisms that indicate an increase in blood glucose is not the primary determinant of satiety following carbohydrate consumption. In studies measuring blood glucose concentrations and satiety using different meals, the satiety mechanism may instead be related to the differing macronutrient content, as protein is considered more satiating than carbohydrate and carbohydrate more satiating than fat (7). Furthermore, foods that delay gastric emptying, such as fat, would be expected to slow the absorption of food, subsequently delaying the return of hunger (38), (39). Gut hormones with the potential to influence satiety are released in response to the presence of food in the small intestine, the quantity of hormones released is dictated by the length of interaction of the carbohydrates in the intestinal tract (7). A longer interaction would be seen with low glycaemic carbohydrates, thus providing yet another mechanism of promoting satiety that does not involve the subsequent change in blood glucose concentrations.

3.5 Isomaltulose (Palatinose)

Isomaltulose (also known by the tradename Palatinose™), like sucrose, is a disaccharide consisting of the monosaccharides glucose and fructose connected via a glycosidic bond.
In contrast to sucrose which has an alpha-1,2 glycosidic bond, isomaltulose has an alpha-1,6 glycosidic bond (40).

![Chemical structure of isomaltulose](image)

**Figure 3.1:** The chemical structure of isomaltulose (40).

Commercial isomaltulose is formed by using glycosyltransferase, which converts the alpha-1,2 bond of sucrose into the alpha-1,6 bond of isomaltulose (41) (**Figure 3.2**). The commercial production of isomaltulose has lead to isomaltulose being used as a sugar in Japan and other Asian countries for more than two decades (9). Although not widely used yet, approval for isomaltulose as a novel food was agreed upon in New Zealand and Australia in 2007 (40). This decision was based on several toxicity studies showing that administration of large doses of isomaltulose did not result in any adverse effects (42), and the gastrointestinal tolerance being comparable to that of sucrose (43). Additionally, it is thought that isomaltulose can provide health benefits, particularly for those with diabetic or pre-diabetic dispositions, due to its low glycemic properties (9).
Figure 3.2: Isomaltulose enzymatic rearrangement (41).

Isomaltulose is approximately half as sweet as sucrose and is naturally found in very low levels in sugar cane juice and honey (40). Its caloric value is 16.7 kJ/g (4kcal/g), the same as that of sucrose (44). The difference is found in the rate at which the two sugars are digested. A three-hour trial observing the glucose and insulin response after consuming a 50g portion of isomaltulose or sucrose found that isomaltulose produced the lowest blood glucose and insulin response, which was more than 50% lower when compared with sucrose (9). A similar study found comparable results when tested on overweight individuals (45). This study also discovered that with lower glucose and insulin responses, postprandial fat oxidation rates were higher, thus having the potential to prevent body weight gain and insulin resistance (45).

3.5.1 Digestion and absorption of isomaltulose

Isomaltulose is completely hydrolyzed and absorbed in the small intestine (unlike sugar alcohols xylitol or sorbitol), although at a slower rate than sucrose (41, 46). Thus, blood glucose and insulin concentrations rise slower and reach a lower peak than after sucrose consumption (42). This slower rise in blood glucose concentrations has been found in both healthy and diabetic individuals (42).
Ileostomy work has been conducted to confirm that isomaltulose is completely digestible. Ten healthy subjects with an ileostomy participated in the study and consumed a test meal containing 50g of isomaltulose on two separate days (9). One test meal was a 500mL beverage and the other was a 250mL beverage with two biscuits, each containing 25g of isomaltulose (9). After collecting and examining the ileostomy bag contents, it was found that digestibility and absorption of the isomaltulose was virtually complete for both test meals, irrespective of the food matrix (9).

During digestion isomaltulose undergoes hydrolysis of its alpha-1,6 glycosidic bond by the sucrase-isomaltase complex (42). This hydrolysis is slower in isomaltulose than sucrose and occurs along the entire small intestine, unlike more quickly absorbed sugars where absorption only takes place in the upper parts of the small intestine (46). The maximal velocity value for the hydrolysis of isomaltulose is only about 26%-45% of that of sucrose’s, due to its more stable alpha-1,6 linkage (43). The slower hydrolysis of isomaltulose is evident when observing the blood glucose and insulin responses of the two sugars (9, 41-43, 45).

### 3.5.2 Isomaltulose and Satiety

In a Japanese study, rats were sustained on distilled water concentrated with 30% isomaltulose or sucrose and subsequent energy intakes were measured on four consecutive days (47). The food and total energy intakes during 24-hours were significantly decreased in the isomaltulose group compared with the sucrose (47). The authors have suggested that this may be due to the isomaltulose group having lower gastric emptying rates and significantly lower blood glucose and insulin concentrations than the sucrose group, suggesting that isomaltulose may be beneficial for appetite.
control (47). However, only eight rats were used making this a small study with little statistical power, and whether or not these findings are relevant to humans is unknown.

There appears to be a number of benefits of using isomaltulose as a sugar alternative, such as a slower rate of digestion resulting in blunted postprandial glucose and insulin responses (41), and an enhanced postprandial fat oxidation rate. The more stable alpha-1,6 bond cannot be broken down by most mouth bacteria and therefore acids that are damaging to the teeth are not produced, making isomaltulose a more tooth friendly alternative to sucrose (46). Additionally, it is classified as a low-GI carbohydrate (GI = 32) (9), proven to improve glycaemic control and blood lipid profiles when compared to high-GI carbohydrates (44). However, there appears to be a gap in the literature that focuses on the satiating properties of isomaltulose compared to sucrose in humans.

3.6 Conclusion and rationale for research

Both obesity and diabetes mellitus have reached epidemic proportions, making these metabolic diseases the leading cause of death and disabilities around the world (48). These rising rates have paralleled the shift from traditional diets to highly processed westernised diets, which are rich in high glycaemic carbohydrates (1). A number of short-term studies suggest that high glycaemic foods are less satiating than low glycaemic foods, which may explain this rise in metabolic disease rates. However, the evidence is inconsistent, likely due to confounding making it difficult to isolate any effect of circulating blood glucose concentrations on satiety.
Therefore, the current study was conducted to investigate the impact of PPG on satiety by using a sucrose (medium-GI) and an isomaltulose (low-GI) beverage, to control for all factors, isolating any effect to a difference in glycaemic response. Our double-blind, randomised controlled trial will be novel as the satiation of isomaltulose compared to sucrose has not been investigated in humans before. Furthermore, satiety will be measured via both VASs and weighed diet records to provide both subjective and objective data. If the claim that low-GI keeps you fuller for longer is supported by the current study (2), isomaltulose used in sugar sweetened beverages could provide a means of reducing excess energy intake, despite containing the same energy as sucrose. In turn, reducing risk of metabolic diseases such as obesity, diabetes and heart disease.
4. **Objective Statement**

The aim of this study was to measure the effects of postprandial glycaemia (PPG) on satiety, using sucrose and isomaltulose sweetened beverages.

The objectives of this study are:

- To develop two isoenergetic beverages containing either sucrose or isomaltulose that are identical in taste and appearance.
- To measure the glycaemic response of the two beverages via finger-prick capillary blood samples.
- To ascertain whether a low-glycaemic index (GI) beverage makes you feel fuller for longer by measuring satiety via visual analogue scales (VASs).
- To ascertain whether a low-GI beverage provides sustained satiety by measuring subsequent energy and macronutrient intake via weighed diet records.
5. **Subjects and Methods**

This study was a double-blinded randomised controlled crossover trial, which ran for four-weeks at the Department of Human Nutrition, University of Otago, Dunedin, New Zealand in March 2018. The main experiment involved participants ingesting two beverages sweetened with different sugars in randomised order, two-weeks apart, and measuring satiety outcomes. In preparation for the main experiment, sensory testing was carried out by six volunteers in order to match the sweetness of the two beverages; and blood glucose testing was carried out by twelve volunteers to quantify differences in the glycaemic responses to the two test beverages.

5.1 **Ethics**

The study was approved by the Human Ethics Committee of the University of Otago in October 2017 (ethics committee number 17/011) *(Appendix A)*. The trial was registered with the Australian New Zealand Clinical Trial Registry *(ACTRN12618000901202)* *(Appendix B)*. All subjects gave written informed consent before entering the study *(Appendix C)*.

5.2 **Participants**

5.2.1 **Recruitment**

Seventy-seven people were invited to participate from a 300-level undergraduate Human Nutrition class from the University of Otago via class presentation. Twelve of these participants volunteered for blood sampling to measure glycaemic response. Prior to these trials six volunteers not involved in the study were recruited for sensory testing of the test beverages via verbal invitation.
5.2.2 Eligibility criteria

Inclusion criteria were all HUNT 311 students aged between 18-60 years old at the University of Otago. Participants were not eligible if they had diabetes or an intolerance to the sweeteners being used. The Joint Expert Committee on Food Additives confirms that sucralose is safe for general food use and an acceptable daily intake of 0.15mg/kg body weight has been established (49). Food Standards Australia New Zealand accepted isomaltulose as a novel food in 2007, declaring it poses no public health safety concern to the majority of consumers (40).

5.2.3 Randomisation

Randomisation of the beverage the participants received each week was computer generated using Microsoft® Excel® for Mac (Microsoft Corporation 2015™, United States of America) by a staff member not involved in the study (Figure 5.1). Once prepared, the two beverages were stored in different refrigerators and were distributed by two members of staff not involved in the study. To ensure double-blinding, the type of sweetener in the beverage was unknown to participants and to those collecting the data.
Figure 5.1: Representation of study design and order of test beverages participants were randomised too.

5.3 Study Design

The study was designed as a double-blinded randomized controlled trial, in which 77 participants were recruited to consume a single 500mL beverage two weeks apart, one of which contained 50g of sucrose and one which contained 50g of isomaltulose and 0.035g of sucralose. Satiety was measured via visual analogue scale (VAS) questionnaires (Appendix D) and weighed diet records. Glycaemic response was measured via finger-prick capillary blood samples on twelve volunteers from the total study participants, after consumption of each of the beverages, two weeks apart. The laboratory timelines are presented in Figures 5.2 and 5.3.

To ensure double-blinding, the drinks had the same volume and appearance and were matched for sweetness using a sensory testing protocol prior to these trials.
5.4 Test beverages

The beverages were made up in 500mL bottles of Pure New Zealand sparkling water. Fifty grams of sucrose (caster sugar, Smart Choice; New Zealand) or isomaltulose (unflavoured Palatinose®, Myprotein; United Kingdom), were measured on calibrated electronic scales (Sartorius, model 1702, Germany) accurate to one hundredth of a gram. Fifty grams of sucrose or isomaltulose were used as that is how much sugar is in a standard 500mL sugar sweetened beverage (50), making it representative of a beverage that the general population would regularly consume.
5.4.1 Composition of test beverages

The composition of each test beverage is shown in Table 5.1. As isomaltulose is approximately half as sweet as sucrose (40), the sweetener sucralose (98% sucralose powder, J66736, lot:T21D050 Alfa Aesar; China) was used in the artificially sweetened beverages. The amount of 0.035g was decided upon based on trial and error and loosely following the sweetening compositions comprising sucralose and isomaltulose patent as a guide (51). To ensure the beverages were both palatable and indistinguishable from each other they were served chilled, and lemon flavouring (Lemon 59223, lot:1002802470, Invita NZ Ltd; New Zealand) and carbonated water were used. Both beverages appeared and tasted identical.

Table 5.1: Composition of test beverages

<table>
<thead>
<tr>
<th>Beverage type (500mL)</th>
<th>Lemon flavour</th>
<th>Sucrose (g)</th>
<th>Isomaltulose (g)</th>
<th>Sucralose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>0.05mL</td>
<td>50.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Isomaltulose</td>
<td>0.05mL</td>
<td>0</td>
<td>50.00</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*Abbreviations: mL = millilitre, g = gram.*

5.4.2 Preparation of test beverages

All food safety principles applicable were adhered to in the preparation of these beverages (52). This included ensuring a hygienic workplace with clean benches, utensils, equipment and washing and drying hands regularly (52). The sucrose and isomaltulose beverages were made up in 500mL bottles of Pure New Zealand sparkling water. Eighty millilitres and 85mLs of the water were measured in volumetric beakers.
and removed from the bottles which sucrose and isomaltulose were going to be added too, respectively. This is due to isomaltulose expanding more, thus taking up more space in the bottle. Fifty grams of sucrose or isomaltulose were measured on calibrated electronic scales in plastic containers the day before the laboratory. These containers were sealed and placed on allocated trays overnight.

Beverages were prepared three hours before commencement of the laboratories to ensure they were chilled upon consumption, by two Master of Dietetics (MDiet) candidates and a laboratory technician. The sugars were added to volumetric beakers and filtered boiling water was added up to 80mL. The mixture was stirred thoroughly to dissolve the sugar. The solutions were then put back into the corresponding bottles and 50uL of lemon flavouring was pipetted into all bottles using a P100 pipette (20-100uL, Gilson; France). Three and a half grams of sucralose was measured on the calibrated electronic scales and dissolved in 100mL of boiling water. One millilitre of this solution was pipetted into the isomaltulose beverages using a P1000 pipette (200-1000uL Gilson; France), as each beverage required 0.035g of sucralose. The bottle lids were screwed back on tightly, inverted four times and put in the refrigerator before consumption. The beverages were prepared on the morning of the laboratories to maintain carbonation.

5.5 Sensory testing

A triangle sensory test was conducted prior to the study at 11:00am on the 21st February 2018 at the Sensory Science Research Centre laboratory, University of Otago. During a triangle sensory test, a panellist is blinded and presented with one different and two alike samples of a product to determine if there is a noticeable difference between them (53).
Six participants not involved in the study were recruited to be panellists to test the similarity of the two beverages.

The beverages being tested were prepared the morning of the sensory testing, using the same procedure that would be used to prepare the beverages for all laboratories. Ten millilitres of these beverages were measured and poured into plastic containers, which were then labelled with a computer-generated number to identify what beverage was in that container. Each participant received a set of three 10mL drinks, four times. The six possible order combinations were randomised across panellists and they were instructed to test the samples from left to right, cleansing the palate with water between each sample. The participants were asked to select which of the three samples was different via a computer questionnaire. The participants were unable to distinguish a difference in the test beverages.

5.6 Glycaemic response laboratories

Glycaemic response and satiety was measured the first and third week of March on the twelve recruited volunteers at the University of Otago Mellor Laboratories. Volunteers were asked to fast for two hours prior to the laboratory, and arrive at the laboratory at 12:00pm to consume their lunch. For standardisation purposes participants were provided eight pieces of sushi purchased from WASABI, the University of Otago’s sushi shop. Each participant chose a filling which remained the same for both laboratories. The sushi was kept refrigerated until being served at 12:00pm. Participants were advised to eat all eight pieces of sushi and were provided a 250mL cup of water which they were free to refill. After consumption of their meal they were free to leave the laboratory with
instruction not to eat or drink (except for water) or undertake strenuous exercise, and were asked to return to the laboratory at 1:45pm.

Baseline blood glucose measurements started at 1:50pm and were finished by 2:00pm. At 2:00pm (baseline) the first VAS questionnaire was completed, after which they were asked to consume their beverage within ten-minutes.

After consumption of the beverage the VAS and blood glucose concentration measurements were taken at: 30, 60, 90, 120, 150 and 180-minutes. Participants were seated apart from toilet visits until 5:00pm when the laboratory finished.

5.6.1 Blood sample collection

In preparation for the blood collection the participants were given heat packs to encourage blood flow to their fingers. The finger being pricked was sanitised using an alcohol wipe (Webcol Alcohol Prep, Covidien, United States of America) and gently massaged to further encourage blood flow to the finger tips. The chosen finger was then pricked off centre, with a 1.5mm X 2.0mm disposable lancet (Contact-Activated Lancet, BD Microtainer, United States of America) and further massaged to coax the blood out. The first drop of blood was wiped off using a non-woven swab (Multisorb, BSN medical limited, United Kingdom). The next 500μL of blood was collected in a microtainer containing anti-coagulant (BD Microtainer® Tube with BD Microgard™ Closure. K2EDTA anticoagulant additive, 250-500μL fill volume, code number 365975; United States of America), which was labelled with the participant’s name and time of collection. These microtainers were inverted eight times to ensure the anticoagulant was
mixed with the blood. Pricked fingers were then cleaned and plastered (BSN Medical, United Kingdom).

The finger-prick procedure was standardised, with the trained assistants taking blood from the same participants at the same time, for both blood taking occasions.

5.6.2 Plasma analysis

To separate the plasma from the red blood cells, the samples were centrifuged at 2500 xG for ten-minutes at room temperature. The plasma was then transferred to a Hitachi cup and stored at -20 degrees Celsius until analysed. Samples were thawed when required for analysis in the Department of Human Nutrition Laboratory. Glucose was analysed on the Roche Hitachi Cobas c311 (Roche, Indianapolis, IN, USA) using enzymatic and enzymatic colorimetric methods (Appendix E). Insulin was analysed using an electrochemiluminescence immunoassay on the Roche Hitachi Cobas e411 (Appendix F).

The glucose and insulin measurement procedures were standardized, with the same laboratory technician measuring blood glucose and insulin concentrations using the same methodology. Samples from both blood taking occasions were analysed together to eliminate intra run variability. Quality control was ensured by analyzing lyophilized standard control serums based on human serum for glucose (PreciControl ClinChem Multi 1 and 2, Roche, Indianapolis) and insulin (PreciControl Universal Level 1 and 1, Roche, Indianapolis) (Appendix G, H).
5.7 Satiety laboratories

The laboratories measuring satiety and cognition took place on the second and fourth Friday of March at the University of Otago Mellor Laboratories. Participants were asked to fast for two-hours before the laboratory and arrive to the laboratory at 12:00pm for lunch. The lunch protocol for the full 77 participants was identical to the glycaemic response laboratories (see glycaemic response laboratories section above).

The first VAS questionnaire was filled in at 2:00pm (baseline), after which participants were asked to consume their beverage within ten minutes. After consumption of the beverage the VAS questionnaire was undertaken at: 30, 60, 90, 120, 150 and 180-minutes. Films relevant to the cognitive testing were playing between these 30-minute intervals. The laboratory finished at 5:00pm.

Questionnaires were pre-printed with student ID’s and placed at allocated seats in stapled order to ensure correct documentation.

5.7.1 Measuring satiety

The VAS was composed of four 100mm lines with words anchored at each end, describing the extreme feelings of hunger, satisfaction, fullness and prospective food intake (29). The questions were: Question one (hunger): “how hungry do you feel?” (Not at all/ never been more hungry); Question two (satisfaction) “how satisfied do you feel?” (Completely empty/ cannot eat another bite); Question three (fullness) “How full do you feel?” (Not at all/ totally full); Question four (prospective food
intake) “how much do you think you can eat?” (Nothing at all/ a lot). These questions were asked in the same order at each time point.

The participants were asked to make a mark on the line corresponding to their feelings of hunger seven times over a period of 180-minutes. The satiety was quantified by measuring the distance in mm from the start of the line to the mark made, providing a value between 0 and 100.

At the first laboratory each participant attended they received electronic kitchen scales (Salter, model 3010, England), a diet record sheet (Appendix I) and asked to measure and record all food and drink they consumed after the laboratory until 12:00am. Detailed instructions on how to complete a weighed diet record were verbally explained to the participants. The weighed diet record was then used to examine whether energy intakes on the day of the satiety test was different between test beverages.

5.7.2 Dietary analysis

Participants were provided detailed instructions on how to enter their weighed diet record on the dietary assessment software Kaiculator (Appendix J). A programme developed by the Department of Human Nutrition at the University of Otago that uses the New Zealand food composition database “NZ FOODfiles” for dietary analyses (54). The entries were analysed to compare the subsequent energy intakes after the two test beverages.

5.8 Demographic and anthropometric questionnaire

On arrival to one of the laboratories attended, before consumption of the sushi, all participants were weighed and measured in a standardized procedure using calibrated
electronic scales (Seca Alpha, model 770, Germany) and a freestanding calibrated stadiometer (Holtain Limited, Britain.). Participants were asked to remove their shoes and jackets. The measurements were used to calculate body mass index (BMI) by dividing weight in kilograms by height in metres squared (kg/m\(^2\)). By the end of the study all participants had completed a single demographic questionnaire with information on sex, date of birth, ethnicity and their recorded weight and height (Appendix K).

Ethnographic data was prioritized according to the Ministry of Health protocol and participants identifying as multiple ethnicities were only represented once (Table 6.1), with Maori taking priority over New Zealand European (55).

5.9 Exercise and intake questionnaires

To check compliance and standardize procedures, glycaemic response participants were asked questions about alcohol consumption on the previous night, food and beverage intake between 10:00am and 12:00pm, food and beverage intake between 12:00pm and 2:00pm and any exercise apart from walking undertaken between 12:00pm and 2:00pm (Appendix L). When measuring satiety and cognition all 77 participants were asked the above questions and additionally, if they had previously seen the documentary being shown that day for cognitive testing purposes. They were also asked if they knew which beverage they had received that day, to ensure the blinding was effective (Appendix M).

5.10 Statistical analysis

5.10.1 Sample size calculation

A sample of 60 was sufficient to detect a clinically relevant 400kJ change in subsequent energy intake with a two-sided alpha of 0.05 and 80% power.
5.10.2 Analysis

The statistical analysis was completed by Dr Jill Haszard, a biostatistician within the Human Nutrition Department at the University of Otago. Statistical analyses of the data were completed using STATA/1C version 13.1 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Blood glucose and insulin concentrations were measured as incremental area under the curve (iAUC) for the twelve participants who undertook this part of the study. VAS results were measured as area under the curve (AUC), as this is the recommended analysis for multiple time points (56). Mean and standard deviation (SD) for each condition were reported. Mean differences between treatments, 95% confidence intervals (CIs) and p-values were calculated using mixed effects regression analysis, with the participant’s ID as a random effect and adjusting for randomized order. Only participants with complete data were included in the analysis.

Differences in mean energy, fat, protein and carbohydrate intake after the laboratories were also assessed using mixed effects regression analysis, further adjusted for sex and BMI. Sensitivity analyses were undertaken with energy intake prior to and during the laboratories.
6. Results

Data from 69 participants were included in the analysis. Figure 6.1 shows the flow of participants through the study. Participants who did not take part on both testing occasions and thus had not consumed one of each beverage, were excluded from the analysis (n= 8).

*The same individual was missing from both laboratories (n=1), therefore missing data from eight not nine people.

Figure 6.1: Study design and flow of participants through the study.
6.1 Demographics

Participant demographics are presented in Table 6.1. The age range of participants was 19 to 39 years old (as of 23rd March 2017), with 87.3% under the age of 24. Of the total number of participants 70.4% had a body mass index (BMI) within the healthy range (18.5-24.9kg/m$^2$), with 2.8% being classified as underweight (<18.5kg/m$^2$) and 26.8% being classified as overweight or obese (>25kg/m$^2$). Participants were predominantly female (81%) and of New Zealand European descent. Glycaemic response study participant demographics are presented in Table 6.2.

Table 6.1: Demographic characteristics of satiety study participants (n= 69)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)$^1$</td>
<td>22.0 (0.7)</td>
</tr>
<tr>
<td>Sex, n (%)$^2$</td>
<td>Female / Male</td>
</tr>
<tr>
<td></td>
<td>56 (81) / 13 (19)</td>
</tr>
<tr>
<td>BMI, kg/m$^2$ (SD)$^1$</td>
<td>23.3 (2.7)</td>
</tr>
<tr>
<td>Ethnicity, n (%)$^2$</td>
<td>NZ European</td>
</tr>
<tr>
<td></td>
<td>42 (61)</td>
</tr>
<tr>
<td></td>
<td>Maori</td>
</tr>
<tr>
<td></td>
<td>4 (6)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
</tr>
<tr>
<td></td>
<td>12 (17)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>11 (16)</td>
</tr>
</tbody>
</table>

$^1$Results presented as mean (SD).

$^2$Results presented as n (%).

Abbreviations: n = number, kg/m$^2$ = kilograms per metres squared.
Table 6.2: Demographic characteristics of glycaemic response study participants (n= 12)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Participants (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)$^1$</td>
<td>21.2 (1.4)</td>
</tr>
<tr>
<td>Sex, n (%)$^2$</td>
<td>Female / Male</td>
</tr>
<tr>
<td></td>
<td>10 (83) / 2 (17)</td>
</tr>
<tr>
<td>BMI, kg/m$^2$ (SD)$^1$</td>
<td>21.9 (3.7)</td>
</tr>
<tr>
<td>Ethnicity, n (%)$^2$</td>
<td>NZ European</td>
</tr>
<tr>
<td></td>
<td>7 (58.3)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
</tr>
<tr>
<td></td>
<td>5 (41.7)</td>
</tr>
</tbody>
</table>

$^1$Results presented as mean (SD).
$^2$Results presented as n (%).

Abbreviations: n= number, kg/m$^2$= kilograms per metres squared.

6.2 Blood glucose and insulin concentrations

Blood glucose and insulin concentrations are presented as the mean incremental area under the curve (iAUC) and standard deviation (SD) in Table 6.3. There was a significant difference in iAUC between the test beverages for both blood glucose and insulin concentrations (P<0.05), with the sucrose beverage producing a higher glycaemic and insulinaemic response.
Table 6.3: Mean (SD) blood glucose and insulin iAUC (n= 12)

<table>
<thead>
<tr>
<th></th>
<th>Sucrose</th>
<th>Isomaltulose</th>
<th>Mean difference (95% CI)</th>
<th>P-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>iAUC of blood glucose</td>
<td>61 (52)</td>
<td>17 (52)</td>
<td>-44 (-70, -18)</td>
<td>0.003</td>
</tr>
<tr>
<td>concentration (mmol.min/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC of insulin</td>
<td>2493 (11540)</td>
<td>609 (1115)</td>
<td>-1883 (-2846, 921)</td>
<td>0.001</td>
</tr>
<tr>
<td>concentration (µIU.min/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ P-value <0.05 considered statistically significant.
Abbreviations: CI= confidence interval, mmol/L= millimol per litre, µIU/L= micro international units per litre.

An illustrative comparison of the mean incremental blood glucose and insulin concentrations of the test beverages from baseline (prior to consumption of the beverage) to 180-minutes after consumption of the beverage is shown in Figures 6.2 and 6.3. By the end of the sampling period (180-minutes) glucose concentrations were lower after the sucrose beverage (-1.9mmol/L) and insulin concentrations were lower after the isomaltulose beverage (-25.6µIU/L).
Figure 6.2: Comparison of mean incremental blood glucose concentration following consumption of the test beverages.

Figure 6.3: Comparison of mean incremental insulin concentration following consumption of the test beverages.
6.3 Satiety

6.3.1 Visual analogue scales

Results from VAS questionnaires assessing hunger, satisfaction, fullness and prospective food intake are presented as mean AUC and SD in Table 6.4. The AUC covers baseline to 180-minutes after consumption of the beverage. There were no significant differences in mean AUC for any VAS question at baseline or following consumption of the test beverages.

For every hour the VAS score decreased on average by 18mm for the satisfaction and fullness questions, and increased on average by 18mm and 16mm for the hunger and prospective food intake questions, respectively.

A sensitivity analysis that included the amount of energy participants consumed before or during the test had no appreciable impact on the effect sizes and significance of these results.
Table 6.4: Mean AUC and SD of VAS questions between the sucrose and isomaltulose sweetened beverages (n= 69)

<table>
<thead>
<tr>
<th>VAS question</th>
<th>Sucrose (mm.min)</th>
<th>Isomaltulose (mm.min)</th>
<th>Mean difference (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger1 (mm.min)</td>
<td>6146 (3153)</td>
<td>6167 (3341)</td>
<td>109 (-443, 661)</td>
<td>0.699</td>
</tr>
<tr>
<td>Satisfaction2 (mm.min)</td>
<td>8395 (3112)</td>
<td>8538 (3360)</td>
<td>29 (-569, 627)</td>
<td>0.924</td>
</tr>
<tr>
<td>Fullness3 (mm.min)</td>
<td>8422 (3348)</td>
<td>8311 (3437)</td>
<td>-91 (-725, 544)</td>
<td>0.780</td>
</tr>
<tr>
<td>Prospective food intake4 (mm.min)</td>
<td>7143 (3569)</td>
<td>7197 (3745)</td>
<td>300 (-318, 919)</td>
<td>0.341</td>
</tr>
</tbody>
</table>

*how hungry do you feel?
*how satisfied do you feel?
*how full do you feel?
*how much do you think you can eat?
*P - value <0.05 considered statistically significant.
Abbreviations: VAS = visual analogues scale, CI= confidence interval.

A visual illustration of the difference in appetite measured by the VAS’s is displayed in Figures 6.4, 6.5, 6.6 and 6.7. The higher the AUC for the VASs expressing hunger and prospective food intake (Figures 6.4 and 6.7) and the lower the AUC for the VASs expressing satisfaction and fullness (Figures 6.5 and 6.6), the higher the feelings of appetite.
Figure 6.4: VAS question; How hungry do you feel?

Figure 6.5: VAS question; How satisfied do you feel?
Figure 6.6: VAS question; How full do you feel?

Figure 6.7: VAS question; How much do you think you can eat?
### 6.3.2 Weight diet records

The mean energy and macronutrient intake taken from participants’ weighed diet records are presented in **Table 6.5**. There were no significant differences in energy, fat, protein or carbohydrate intake following ingestion of the two test beverages.

A sensitivity analysis that included the amount of energy participants consumed before or during the test had no appreciable impact on the effect sizes and significance of these results.

**Table 6.5:** Mean (SD) energy and macronutrient intake from weighed diet records (n= 61)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Sucrose</th>
<th>Isomaltulose</th>
<th>Mean difference (95% CI)</th>
<th>P-value$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>4059 (2396)</td>
<td>3768 (2330)</td>
<td>-291 (-845, 267)</td>
<td>0.306</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>41 (26)</td>
<td>34 (27)</td>
<td>-6.6 (-13.3, 0.2)</td>
<td>0.056</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>41 (26)</td>
<td>39 (24)</td>
<td>-2.3 (-8.8, 4.3)</td>
<td>0.498</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>100 (88)</td>
<td>102 (72)</td>
<td>1.6 (-17.2, 20.5)</td>
<td>0.864</td>
</tr>
</tbody>
</table>

$^1$ Adjusted for BMI and sex.
$^2$ P-value $<$0.05 considered statistically significant.
*Abbreviations: CI=confidence interval, kJ= kilojoule, g= gram.*
7. Discussion

The aim of the study was to determine the effect of postprandial glycaemia (PPG) on satiety using an isomaltulose sweetened beverage compared to a sucrose sweetened beverage within a healthy New Zealand population. By design it was confirmed that participants’ glycaemic and insulinaemic responses were lower following ingestion of the isomaltulose compared with the sucrose beverage. The crossover design allowed an individual comparison of glycaemic response, important for eliminating inter-individual variation (13). Despite the differences in postprandial glycaemic and insulinaemic responses, there were no significant differences in either subjective satiety assessed using visual analogue scales (VASs) or by an objective measure of subsequent food intake.

When comparing the current study to similar designed studies results are consistent. This is likely attributed to the double-blinding that eliminates participant and researcher bias and the crossover design which allows for participants to be their own control. Two comparable studies measured subjective appetite and energy intake for three and four-hours after consumption of low, intermediate and high-glycaemic index (GI) beverages (57, 58). Test beverages included a glucose, full milk, and half glucose, half milk beverage and a fructose, glucose, and whey protein beverage, all of which were 1100kJ (57, 58). Despite significant changes in glycaemic response to the beverages in these double-blind, randomised crossover trials, no differences in subjective appetite via VASs or ad libitum energy intake was observed (57, 58).

Generalisability of finding no difference in satiety despite changes in PPG within young healthy adult participants is broadened because Brindal and colleagues used children participants and Bowen and colleagues used overweight participants (57, 58).
Our study’s lack of effect on satiety despite differences in PPG is not in accord with the glucostatic mechanism of food intake proposed by Mayer (6). The glucostatic theory states that temporary increases in blood glucose concentrations correspond to a decrease in food intake and vice versa (6). A study comparing short-term (one-hour) satiety after consumption of high and low-GI isoenergetic beverages, found that the high-GI beverage kept participants fuller at 60-minutes, in accordance with the glucostatic theory (59). It was thus hypothesised that high-GI keeps you fuller short-term and low-GI may sustain satiety long-term (59). However, our findings did not support this hypothesis or show any relationship between blood glucose concentrations and satiety.

In contrast, differences in satiety have been found in other studies that also compared low and high glycaemic treatments. One such study conducted in obese adolescent males comparing ad libitum food intake after low, intermediate or high-GI meals, reported voluntary energy intake after the high-GI meal was 81% greater than after the low-GI meal (60). However, the low-GI meal contained more protein, more fat and less carbohydrate than the high-GI meal (60). Furthermore, the high-GI meal was mainly liquid, compared with the predominantly solid low-GI meal that required chewing (60). Evidence suggests that solid food is more satiating than liquids (57, 61, 62), and that chewing elicits a higher degree of satiety due to alterations in gut hormone responses (63). A randomised crossover study fed participants a breakfast meal with 50g of available carbohydrate with various GI values and energy and macronutrient contents, and found no relationship between glycaemic response and short-term appetite (64). The low-GI breakfast meal was associated with reduced energy intake at lunch, but subsequent energy intake may have been influenced by the fact the low-GI meal
contained a higher energy content (64). A randomised controlled trial that measured glycaemic response and satiety after consumption of a low-GI carob or high-GI chocolate cookie, reported increased satiety after the low-GI cookie (65). However, the low-GI carob cookie contained 9.60 more grams of dietary fibre and 9.15 more grams of monounsaturated fats, due to the carob cookie containing 23g of ground hazelnuts, which the chocolate cookie did not (65). These studies indicate that low opposed to high-GI foods and beverages result in greater satiety (60, 64, 65). However, it is likely that factors other than glycaemic response such as fibre, macronutrient and energy content, contributed to, or were the cause of, the differences in satiety found.

Confounding factors are particularly prominent in long-term studies, thus how PPG effects health over an extended period of time is not well understood. There appears to be little relationship between the GI of foods and weight gain over periods of months and years (27, 32, 66). This is likely attributable to the GI of foods being unrelated to the energy density of foods, for example cake and apples have comparable GI’s but very different energy densities (30). However, some studies have reported a reduction in heart disease and improvement in glycaemic control through following a low glycaemic diet long-term in overweight and insulin resistant individuals (1, 22, 67, 68). Furthermore, it is not clear whether these benefits are independent of the effects of the dietary fibre present in the low glycaemic foods consumed (68).

An acute feeding study that fed participants isoenergetic portions of food with differing GI’s suggests that food volume or the energy density, is the strongest predictor of satiety (30). Our findings support this hypothesis as both beverages were isovolumetric and isoenergetic, however despite a difference in glycaemic response no difference in
VAS scores or ad libitum intake was observed. By using a beverage, we were able to keep energy density the same whilst controlling macronutrient and energy content. However, the liquid form of our treatments may have reduced overall feelings of satiety compared with solid foods (57, 61, 62). This could be attributable to fluids being ingested more quickly, an increased rate of gastric emptying, lack of gastric distention, the act of swallowing without chewing, and potential cognitive bias of solid foods having a perceived higher energy intake (62, 69). Nevertheless, the finding of a lack of difference in feelings of satiety following treatments having differing glycaemic potential is important, especially as consumption of energy-dense sweetened beverages is a major public health concern (70).

Glucose and fructose have been found to influence satiety and metabolic markers differently, despite having the same caloric value and displaying a similar pattern of weight gain over a period of 10-weeks in overweight and obese subjects (71). When assessing the metabolic effects of fructose or glucose sweetened beverages, glucose consumption reduced cerebral blood flow in regions of the brain responsible for appetite and reward processing, when compared to fructose (71-73). Thus, the greater activation of brain regions following fructose consumption has been thought to promote feeding behaviour (73). It can therefore be hypothesised that there are additional mechanisms of satiety after sweetened beverage consumption independent of the glycaemic response. A benefit of using isomaltulose and sucrose as comparison treatments is that identical monosaccharides were involved.

The present study is the first to assess the satiating properties of isomaltulose compared to sucrose in humans. A Japanese study comparing the satiation of sucrose to
isomaltulose in rats found that isomaltulose was significantly more satiating when the sugars were consumed in liquid form (47). These results were not in agreement with the present study, indicating that studies where isomaltulose has been tested in animals may not be correlated well to humans.

7.1 Clinical implications

The results from the current study suggest that despite differences in PPG there is no difference in satiety following the ingestion of sucrose and isomaltulose sweetened beverages. Those with diabetes mellitus may benefit from consumption of the isomaltulose beverage, in comparison to the sucrose beverage, due to its low and sustained effect on blood glucose concentrations, thus minimizing hyperglycaemia which is independently associated with cardiovascular disease (CVD), retinopathy, certain cancers and other serious complications in those with diabetes (12). However, isomaltulose has the same caloric value as sucrose (9) and consumption should be limited in those who are obese or overweight.

Sugary beverages are a significant source of energy in the Western diet and possess no beneficial nutritional properties (61). High intakes are associated with obesity, type 2 diabetes, gout, coronary heart disease (CHD), and CVD, and thus should be limited (70). Most studies that have demonstrated the health benefits of low glycaemic foods involved natural and minimally processed carbohydrate containing cereals, vegetables and fruit (18). These naturally occurring low-GI foods have qualities independent of their GI that are beneficial for our health, such as fibre. Thus, the claim that low-GI keeps you fuller for longer (2), could be attributed to the varying properties of these low-GI foods and not the GI itself. Food choices should not be made solely on
the GI of a food, as processed foods can possess a low-GI whilst also having a substantial amount of sugar and undesirable fat (18).

7.2 Strengths and limitations

The study used a sample of healthy, young nutrition students, limiting the applicability of the findings to the wider population. However, the use of a crossover study design strengthened this study as participants acted as their own control, and double-blinding eliminated bias with preconceived ideas of low-GI keeping you fuller for longer. The use of a beverage made double-blinding easy whilst allowing for control of volume, monosaccharide composition and macronutrient and energy content.

An important aspect of this study was using both VASs and weighed diet records to measure satiety, as although VASs are a validated tool, they only measure subjective feelings of hunger (29). Furthermore, VASs have been found to be less accurate for finding significant differences when compared to weighed diet records (8). The use of a 500mL beverage with 50g of sugar was used as it is relative to commonly consumed sugar sweetened beverages (50), making this study relevant to the real world. A novel aspect of this study was that it was conducted in the afternoon, as studies measuring satiety are commonly conducted in the morning (57, 59, 60, 64, 73, 74), when the impact of satiety is just as important in the afternoon for reducing daily energy intake and preventing weight gain. However, we may have been able to generate a larger difference in glycaemic response if the study had been conducted in the morning after an overnight fast, which may have had a more pronounced effect on satiety. Nonetheless, the use of a beverage without fat or protein, both known to dampen glycaemic response (75), allowed us to maximize glycaemic response with our afternoon study design.
An unavoidable limitation of this study is the use of self-reported data. Participants may not have read the VASs correctly, in turn not accurately marking their feelings of appetite. Additionally, participants could have fabricated their weighed diet records or not entered their data correctly onto the dietary analysis software. To strengthen the study, a placebo could have been used to compare the satiation of the two test beverages too. The energy dense test beverages, may have not affected satiety compared to a non-caloric sweetened beverage, adding empty calories and promoting weight gain. Thus, a study comparing sucrose and isomaltulose to a placebo may be an area for further research.

7.3 Conclusion and recommendations

In a healthy, young cohort of participants, glycaemic response varied significantly after consumption of a sucrose and isomaltulose beverage. However, no significant differences were found when measuring satiety using VASs and weighed diet records to analyse subsequent energy intake. From these results, we can infer that there was no relationship between glycaemic response and satiety in this study. For those with or at risk of diabetes or overweight or obese, it would be sensible to recommend limiting all sugar sweetened beverage consumption, regardless of the GI, and choose wholesome foods that induce a higher degree of satiety.
8. Application to Practice

Part A: the applicability and relevance of the research to dietetic practice

Despite the differences in postprandial glycaemia (PPG) after consumption of the sucrose and isomaltulose beverages, there were no significant differences in either subjective satiety assessed using visual analogue scales (VASs) or subsequent food intake. A possible reason for this finding could be the liquid form of our treatments, as liquids are less satiating than solids (57, 61, 62), and that the beverages contained no additional nutritional properties that would promote satiety.

This research has challenged the common misconception about low-glycaemic index (GI) keeping you fuller for longer and has confirmed that this does not apply to beverages made with isomaltulose. When advising future clients, I will be cognisant of this fact and take extra care when advising on the suitability of low-GI meals, by suggesting low-GI foods rather than beverages, whilst ensuring those low-GI foods are nutrient dense and known to be associated with increased satiation.

As a dietitian, I would be careful in my advice to patients who have diabetes or are on weight loss diets, regarding isomaltulose use. Due to the low glycaemic response of isomaltulose, it may be considered as a sugar alternative to those with diabetes. However, it contains the same energy content as sucrose (9) and thus should be limited in those who are either obese or overweight.

Undertaking this research has also given me the opportunity to learn other beneficial skills such as how to critically review and evaluate the literature. This will be invaluable when working as a dietitian in using evidence based literature to keep me up to date with the latest research.
Part B: Reflective Practice: What this research experience has meant to me

An aspect of my research journey that stands out as being particularly important for my growth and development as a dietitian, was working alongside my Master of Dietetics (MDiet) research partner (CK). I was initially hesitant to be undertaking a research project with a partner as I have always been an independent worker. However, working with CK proved to be incredibly beneficial and actually far more enjoyable than I initially anticipated. I quickly adapted and learned how to work as an efficient team member, contributing ideas and discussing solutions to problems openly. This was particularly evident in designing and implementing the experimental phase of the study, as there were some methodological issues that occurred that CK and I had to be quick to resolve.

Firstly, we ran out of specific ingredients needed for making the beverages, requiring us to get in touch with multiple members of staff who were responsible for ordering and finances. However, it also required us to implement a back up plan in case the supplies did not arrive in time. Secondly, due to participant error we had to alter the protocol for the second laboratory testing session to keep methodological procedures the same, thus ensuring the tests were comparable. When faced with these problems we worked together to resolve them, teaching us how to be adaptable under pressure and improving our problem solving skills immensely.

This positive experience working alongside CK prompted me to become a member of the MDiet thesis writing group. This group met weekly and implemented a feedback system where we would read sections of each other’s thesis chapters and provide feedback. It was advantageous to not only read colleagues’ work but also to get feedback to help improve the clarity in my writing of this thesis.
By working so closely with CK and other MDiet colleagues, I believe I have gained outstanding teamwork, communication, problem solving and organizational skills. Although I had to regularly communicate with lots of different people (supervisors, laboratory technicians, biostatistician) throughout this process, I continued to work alongside CK for the duration of the research and write up. By working in partnership with CK, we continued to communicate our ideas, and resolve problems whilst still remaining highly organized during this stressful time. These skills will have a lifelong positive impact on my future career as a dietitian.
9. References

65. Papakonstantinou E, Orfanakos N, Farajian P, Kapetanakou AE, Makariti IP, Grivokostopoulos N, et al. Short-term effects of a low glycemic index carob-
10. Appendices

Appendix A: Ethics proposal and approval letter
Appendix B: Australian New Zealand Clinical Trial Registry
Appendix C: Consent form
Appendix D: VAS satiety questionnaire
Appendix E: Enzymatic and enzymatic colorimetric methods
Appendix F: Electrochemiluminescence immunoassay methods
Appendix G: PreciControl ClinChem Multi 1 and 2
Appendix H: PreciControl Universal
Appendix I: Diet record sheet
Appendix J: Kaiculator instructions
Appendix K: Demographic and anthropometric questionnaire
Appendix L: Exercise and intake questionnaire: glycaemic response laboratories
Appendix M: Additional information questionnaire: satiety laboratories
Appendix A: Ethics proposal and approval letter

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 request for the amendment to give students a sweet beverage containing 50mg of sucralose instead of a “trifle”. Thank you for keeping the Committee informed.

Your proposal continues to be fully approved by the Human Ethics Committee. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing. I hope all goes well for you with your upcoming research.

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: Australian New Zealand Clinical Trial Registry

Trial ID
ACTRN12618000901202
Ethics application status
Approved
Date submitted
25/05/2018
Date registered
29/05/2018
Date last updated
Type of registration
Retrospectively registered

Public title
Effect on satiety and cognitive function following the ingestion of beverages containing sucrose or isomaltulose by healthy adults

Scientific title
The effect in healthy adults of consuming sucrose or isomaltulose sweetened beverages on measures of satiety and cognitive function

Universal Trial Number (UTN)
U1111-1214-7109

Health condition(s) or problem(s) studied:
Cognition
Satiety

Condition category
Condition code
Diet and Nutrition

Other diet and nutrition disorders
Metabolic and Endocrine
Normal metabolism and endocrine development and function

Intervention/exposure
Study type
Interventional

Description of intervention(s) / exposure
This will be a double-blind crossover trial in which 75 healthy adults will ingest a 500ml sparkling water beverage containing either 50g of sucrose or a beverage containing a mix of 50g isomaltulose with 45mg sucralose. The order in which participants receive the beverages will be randomised to each person. There will be a minimum 2 day washout between beverages. Prior to the intervention, participants will be given a standard lunch of sushi and water. Participants will ingest the intervention beverages within 15 minutes 1.5hr following lunch. Eating lunch and subsequent assessment of satiety and tests of cognition will be under the supervision of the study investigators.

Intervention code [1]
Treatment: Other
Comparator / control treatment
This is a crossover trial with the sucrose beverage used as the comparator
Control group
Active

Outcomes
Primary outcome [1]
Satiety assessed via the use of visual analogue scales (Likert) in response to four appetite questions (subjective).
Timepoint [1]
Subjective satiety will be quantified at baseline and at 30, 60, 90, 120 and 150 minutes following beverage ingestion.

Primary outcome [2]
A composite outcome comprising a battery of tests of cognitive function (word recall; audio-visual memory; trailmaking)
Timepoint [2]
Tests of cognition will be quantified at 45, 90 and 135 minutes following beverage ingestion.

Primary outcome [3]
Satiety assessed as subsequent energy intake
Timepoint [3]
Food and beverages ingested from 12pm to 12am of each test day

Secondary outcome [1]
Postprandial monitoring of blood glucose concentrations over a period of 3h following beverage ingestion.
Timepoint [1]
Capillary blood will be sampled via fingerprick at baseline at 30, 60, 90, 120 and 150 minutes following beverage ingestion.
Eligibility
Key inclusion criteria
Healthy adults
Minimum age
18 Years
Maximum age
75 Years
Gender
Both males and females
Can healthy volunteers participate?
Yes
Key exclusion criteria
Intolerance to isomaltulose or sucralose

Study design
Purpose of the study
Treatment
Allocation to intervention
Randomised controlled trial
Procedure for enrolling a subject and allocating the treatment (allocation concealment procedures)
All participant names will be entered into a dataset. A random number generator will be used to generate a random number next to each participant. The dataset will be sorted in ascending random number order. On the first test day, the first 37 participants in the sorted dataset will be allocated one treatment and the last 38 participants the alternative treatment; on the second test day, the treatments will be reversed (crossover).
Randomisation and the supply of beverages to participants will be undertaken by a University staff member otherwise uninvolved in the study. Allocation concealment was achieved by central randomisation by computer.
Methods used to generate the sequence in which subjects will be randomised (sequence generation)
Simple randomisation using a randomisation table created by computer software
Masking / blinding
Blinded (masking used)
Who is / are masked / blinded?
The people receiving the treatment/s
The people administering the treatment/s
The people assessing the outcomes
The people analysing the results/data
Intervention assignment
Crossover
Other design features
Phase
Not Applicable
Type of endpoint(s)
Efficacy
Statistical methods / analysis
Using published data and assuming a within-person correlation of 0.5, a sample size of 70 is sufficient to detect a difference of 0.5 SD for the quality of memory, trailmaking and speed of attention tests using ANCOVA (Scholey et al., 2004). For satiety, 70 participants will have 80% power to detect a 5mm difference in VAS scores between trifles (Flint et al., 2000). To allow for dropout, 75 people will be recruited.

Scholey, AB and Kennedy, DO, Cognitive and physiological effects of an "energy drink": an evaluation of the whole drink and of glucose, caffeine and herbal flavouring fractions, Psychopharmacology (Berl), 2004; 176: 320-30.


Recruitment
Recruitment status
Completed
Date of first participant enrolment
Anticipated
Actual
1/03/2018
Date of last participant enrolment
Anticipated
Actual
8/03/2018
Date of last data collection
Anticipated
Actual
6/04/2018
Sample size
Target
75
Accrual to date
Final
75
Ethics approval number [1]
17/011

Summary
Brief summary
It has been postulated that satiety and cognitive function are dependent upon the concentration of circulating blood glucose. The primary purpose of the study therefore, is to test whether measures of satiety and cognition are effected by differing concentrations of circulating blood glucose concentrations. To generate differences in blood glucose
concentration, beverages sweetened with 50g of either sucrose or isomaltulose were developed. In order to match the beverages for sweetness, a small amount of sucralose was added to the isomaltulose beverage. A triangle taste test was undertaken in six people with the result that the two beverages were indistinguishable from one another. The blood glucose response to the test beverages was undertaken in a subset of 12 of the 75 participants. The tests of satiety and cognition were conducted in the afternoon. The order in which participants received the test beverages was randomised to each person. Each participant consumed both of the test drinks in a crossover design with a washout of at least two days. Standard test methodology was used to test for subjective and objective satiety. During the test period of three hours, a film was shown in three half hour time-slots with a 20 minute interval between each showing. During the three intervals, a standard word recall test was administered and participants answered questions relating to the section of film that had just been screened. One trailmaking test was administered at the end of each session. The people administering the tests and the participants were unaware of which beverage had just been consumed (double-blinding). Data analysis was undertaken by a biostatistician blinded to order of treatment. The study hypothesis was that a more stable blood glucose concentration over time, represented by the isomaltulose treatment, would result in greater satiety and a better cognitive performance.

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Fax
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bernard.venn@otago.ac.nz
Appendix C: Consent form

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 beverages sweetened with sucrose or isomaltulose+sucralose 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 no commercial use will be made of the data.

12. For people providing blood, I understand that the 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 D: VAS satiety questionnaire

ID…………………..

Date ……………

Time ……………

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 E: Enzymatic and enzymatic colorimetric methods

**GLUC3**

**Glucose HK**

<table>
<thead>
<tr>
<th>Order information</th>
<th>Cat. No.</th>
<th>System-ID 70 683I 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose HK</td>
<td>04464483</td>
<td>190</td>
</tr>
<tr>
<td>Calibrator f.a.s. (12 x 3 mL)</td>
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<td>190</td>
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<td>Calibrator f.a.s. (12 x 5 mL, for USA)</td>
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<td>360</td>
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<tr>
<td>Precinorm U plus (10 x 3 mL)</td>
<td>12149435</td>
<td>122</td>
</tr>
<tr>
<td>Precinorm U plus (10 x 3 mL, for USA)</td>
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<td>160</td>
</tr>
<tr>
<td>Precipath U plus (10 x 3 mL, for USA)</td>
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<td>05517216</td>
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</tr>
<tr>
<td>Diluent Na+ 9 % (% (50 mL)</td>
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<td>190</td>
</tr>
</tbody>
</table>

### English

**System information**

For **cobas c 311/501** analyzers:

**GLUC3**: ACN 717

**SOLU**: ADN 668 (STAT, reaction time: 7)

For **cobas c 502** analyzer:

**GLUC3**: ACN 8717

**SOLU**: ADN 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**

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, hypoglycemia or insulin-induced hypoglycemia. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glycemia, 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.

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

\[
\text{Glucose + ATP} \rightarrow \text{G-6-P + ADP}
\]

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 NADP+ formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

\[
\text{G-6-P + NADP+} \rightarrow \text{G-6-PD} \rightarrow \text{gluconate-6-P + NADPH + H+}
\]

### Reagents - working solutions

R1: MES buffer: 5.0 mmol/L, pH 6.0; MgCl2: 24 mmol/L, ATP: ≥ 4.5 mmol/L, NADP+: ≥ 7.0 mmol/L, preservative

R2: HEPES buffer: 200 mmol/L, pH 6.0; MgCl2: 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 Na+ 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: Lip-heparin, K2-EDTA, NaF-Na2EDTA, KF-Na2EDTA, NaF-KOxalate.

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 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.

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
Appendix F: Electrochemiluminescence immunoassay methods

**Insulin**

**Insulin**

<table>
<thead>
<tr>
<th>E170</th>
<th>cobas e 411</th>
<th>cobas e 601</th>
<th>cobas e 602</th>
</tr>
</thead>
<tbody>
<tr>
<td>12007547 122</td>
<td>• Indicates analyzers on which the kit can be used</td>
<td>100 tests</td>
<td>100 tests</td>
</tr>
</tbody>
</table>

**English**

**Intended use**

Immunosay for the in vitro quantitative determination of human insulin in human serum and plasma. The determination of insulin is utilized in the diagnosis and therapy of various disorders of carbohydrate metabolism, including diabetes mellitus and hypoglycemia.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

**Summary**

Insulin is a peptide hormone with a molecular weight of approximately 6000 daltons. It is secreted by the B-cells of the pancreas and passes into circulation via the portal vein and the liver. Insulin is generally released in pulses, with the parallel glucose cycle normally about 2 minutes ahead of the insulin cycle. The insulin molecule consists of two polypeptide chains, the A-chain with 21 and the B-chain with 30 amino acids. Biosynthesis of the hormone takes place in the B-cells of the islets of Langerhans in the form of single-chain proinsulin, which is immediately cleaved to give proinsulin. Specific proteases cleave proinsulin to insulin and C-peptide which pass into the bloodstream simultaneously. About half of the insulin, but virtually none of the C-peptide, is retained in the liver. Circulating insulin has a half-life of 3-5 minutes and is preferentially degraded in the liver, whereas inactivation or excretion of proinsulin and C-peptide mainly takes place in the kidneys.

The amino acid sequence of insulin has remained surprisingly constant during evolution, with the result that prior to the development of genetically engineered human insulin it was possible to successfully use porcine or ovine insulin in the therapy of diabetes mellitus.

The action of insulin is mediated by specific receptors and primarily consists of facilitation of the uptake of sugar by the cells of the liver, fatty tissue and muscle; this is the basis of its hypoglycemic action.

Serum insulin determinations are mainly performed in patients with symptoms of hypoglycemia. They are used to ascertain the glucone/insulin quotients and for clarification of questions concerning insulin secretion, e.g. in the tubularastad test and glucagon test or in the evaluation of oral glucose tolerance tests or hunger provocation tests.

Although the adequacy of pancreatic insulin synthesis is frequently assessed via the determination of C-peptide, it is still generally necessary to determine insulin. For example, therapeutic administration of insulins of non-human origin can lead to the formation of anti-insulin antibodies. In this case, measurement of the concentration of serum insulin shows the quantity of free - and hence biologically active - hormone, whereas the determination of C-peptide provides a measure of the patient's total endogenous insulin secretion. A disorder in insulin metabolism leads to massive influencing of a number of metabolic processes. A too low concentration of free, biologically active insulin can lead to the development of diabetes mellitus. Possible causes of this include destruction of the B-cells (type I diabetes), reduced activity of the insulin or reduced pancreatic synthesis (type II), circulating antibodies to insulin, delayed release of insulin or the absence (or inadequacy) of insulin receptors. On the other hand, autonomous, non-regulated insulin secretion is generally the cause of hyperglycemia. This condition is brought about by inhibition of gluconeogenesis, e.g. as a result of severe hepatic or renal failure, islet cell adenoma, or carcinoma. Hyperglycemia can, however, also be facilitated intentionally or unintentionally (facilitous hyperglycemia).

In 3 % of persons with reduced glucose tolerance, the metabolic state deteriorates towards diabetes mellitus over a period of time. Reduced glucose tolerance during pregnancy always requires treatment. The clearly elevated risk of mortality for the fetus necessitates intensive monitoring.

The Elecsys Insulin assay employs two monoclonal antibodies which together are specific for human insulin.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex \( R \) form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCellProCell M.
- Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

**Reagents - working solutions**

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
- Streptavidin-coated microparticles 0.72 mg/mL, preservative.
- Anti-insulin-Ab–bixin (gray cap), 1 bottle, 10 mL:
  - Biotinylated monoclonal anti-insulin antibody (mouse) 1 mg/L,
  - MES buffer 50 mmol/L, pH 6.0, preservative.
- Anti-insulin–Ab–Ru(bipy) \( R \) (black cap), 1 bottle, 10 mL:
  - Monoclonal anti-insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L,
  - MES buffer 50 mmol/L, pH 6.0, preservative.

**Precautions and warnings**

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional use on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators, and controls).

**Reagent handling**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

**Storage and stability**

Store at 2-8 °C.

Store the Elecsys Insulin reagent kit upright in order to ensure complete container of the microparticles during automatic mixing prior to use.

Stability:

- unopened at 2-8 °C: up to the stated expiration date
- after opening at 2-8 °C: 12 weeks
- on the analyzers: 4 weeks

**Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

- Lip-heparin, K\( _{3} \) EDTA, and sodium citrate plasma.

Hemolysis interferes, as insulin-degrading peptidases are released from erythrocytes.

**Criteria**

Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within \( < x \) x analytical sensitivity (LDL) \( x \) coefficient of correlation > 0.95.

Specific for 24 hours at 2-8 °C, 6 months at -20 °C. Freeze only once.

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

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
Appendix G: PreciControl ClinChem Multi 1 and 2

510(k) Summary – PreciControl ClinChem Multi 1 and 2

Introduction
Roche Diagnostics Corporation hereby submits this 510(k) to provide notification of our intent to market new controls named PreciControl ClinChem Multi 1 and 2.

Submitter name, address, contact
Roche Diagnostics
9115 Hague Rd.
PO Box 50416
Indianapolis, IN 46250
Phone: (317) 521-3954
Fax: (317) 521-2324
Contact Person: Patrick Stimart
Date prepared: July 16, 2010

Device Name(s)
Proprietary name(s): 1. PreciControl ClinChem Multi 1 and 2
Common name(s): PCCC Multi 1 and 2
Classified under 21 CFR 862.1660
Classification name(s): Multi-analyte controls, all kinds (assayed and unassayed)
Product Code: JJY

Device Description
The PreciControl ClinChem Multi 1 and 2 are quality control products consisting of lyophilized human sera with constituents added as required to obtain desired component levels. Concentrations of the components in the controls have been adjusted to represent normal and pathological levels. The concentrations of the components in the controls are lot-specific and representative values are given in the enclosed value sheets.

Continued on next page
Appendix H: PreciControl Universal

510(k) Summary – PreciControl Universal

Introduction
According to the requirements of 21 CFR 807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitter
Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250
317-521-3723
Contact Person: Gail Sauers
Date Prepared: February 27, 2009

Submission purpose
Roche Diagnostics hereby submits this Traditional 510(k) device modification to provide notification of changes to our control material, Elecsys PreciControl Universal (PCU). PreciControl Universal is used for quality control of Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers. This product contains control material for numerous Elecsys assays in one convenient solution. Changes to PCU consist of the addition of Carcinomembran antigen (CEA) control and total (free + complexed) Prostate-Specific Antigen (PSA) to extend the current functionality.

Device Name
Proprietary name: Elecsys PreciControl Universal.
Common name: PreciControl Universal
Classification name: Multi-Analyte Controls, All Kinds (assayed and Unassayed)

Device Description
The Elecsys PreciControl Universal is a lyophilized product consisting of added antigens in a human serum matrix. During manufacture, the analytes are spiked into the matrix at the desired concentration levels.

Intended use
Elecsys PreciControl Universal is used for quality control of Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers.

Continued on next page
Appendix I: Diet record sheet

ID:

HUNT311 Glycaemic Laboratory

<table>
<thead>
<tr>
<th>Time</th>
<th>Amount</th>
<th>Food or drink</th>
<th>PTO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
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</tr>
<tr>
<td>24:00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PTO – if you need more space to write your foods and drinks please tick at the appropriate time and continue over page
Appendix J: Kaiculator instruction

Logging on:
Open the Firefox or Chrome web browser
Type https://sybil.otago.ac.nz/dietary/opening.das? in 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.

How to enter a diet within a project:
Select the project e.g. HUNT311 > select Diets > select Records > select New

Set up the record:
Record the Record ID number, usually the study participant ID number. Kai-culator produces a default ID RRRXXX but overide this with your chosen unique ID e.g. your name or student ID to make up 6 letters with no spaces in between. If students share the same name, e.g., four students named ‘Alex’ ensure your Record IDs are all different.

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 (liz.fleming@otago.ac.nz). Send her the incorrect ID and the new correct ID. Liz can not change the Record IDs if students use the same ID. You will have to enter your diets again under another unique Record ID.

- Select Day# and Type in 1 or subsequent day number
- Select date that diet was recorded from drop down menu
- Select Start
- 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’ and then 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.
• 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 does not work on the left hand box when typing in quantities.** You have to enter liquids on the middle box and select mls as the unit and then type in e.g. 180 for 180ml.

<table>
<thead>
<tr>
<th>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’</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Frequently the first word is a generic name such as ‘bread’ or ‘beef’</td>
</tr>
<tr>
<td>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.</td>
</tr>
<tr>
<td>c) If ‘Starts with’ does not find the food you are looking for choose ‘Contains’ from the drop down list and click search.</td>
</tr>
<tr>
<td>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)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hints</th>
</tr>
</thead>
<tbody>
<tr>
<td>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 <strong>Measure descriptor</strong> ‘How many’ column takes priority over a value in the <strong>Volume ‘How many’ field.</strong> which takes priority over a gram value in the <strong>Amount column</strong></td>
</tr>
<tr>
<td>b) Click the &quot;Save&quot; icon (far left icon below the Food diary) to commit these changes.</td>
</tr>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

**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 only suitable 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 2018
  • Click Composition Data>Recipes
  • Click the + icon
  • Type in the name of the recipe > 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 (100°C for boiled, steamed, microwaved; 180°C for baked, roasted, stir-fried, fried. 220°C 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**

**Open a second browser, e.g., if you are already using Firefox open Chrome**

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...
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. Click ‘Save’
- Type in the name of the second ingredient > Click OK repeat for all ingredients.
- 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.

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

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.
Click the ‘Amount field’. Enter the amount in the ‘g’ amount field, or choose a measure description or choose a volume and enter the dimensions of the food.

Repeat the last two steps for all ingredients

Click ‘Save recipe’ 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, e.g., 100% or 50% etc > Click OK

Click ‘Save’ icon. Click ‘Exit’ icon

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’

- Frequently the first word is a generic name such as bread or beef
- 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.
- If the ‘Starts with’ field does not find the food you are looking for choose ‘Contains’ from the drop down list and click search.
Appendix K: Demographic and anthropometric questionnaire

What is your date of birth? (DD/MM/YYYY)

Are you female or male? (please circle)

What ethnicity do you affiliate with?

Which ethnic group do you belong to? Mark the space or spaces that apply to you.

- New Zealand European
- Māori
- Samoan
- Cook Island Māori
- Tongan
- Niuean
- Chinese
- Indian
- Other (such as Dutch, Japanese, Tokelauan). Please state:

Weight: __________ kg

Height: __________ cm

Thanks for completing the questionnaire!
Appendix L: Exercise and intake questionnaire: glycaemic response laboratories

ID:

1. Did you consume any alcohol last night?
   Please circle YES / NO
   If yes how many standard drinks?

2. Did you consume any beverages apart from water or food between 10am and 12pm today?
   Please circle YES / NO
   If yes what was it and how much? Please provide serving sizes eg medium apple, 1 cup.

3. Did you consume any beverages apart from water or food between 12 and 2pm?
   Please circle YES / NO
   If yes what was it and how much? Please provide serving sizes eg medium apple, 1 cup.

4. Did you do any exercise apart from walking between 12 and 2pm?
   Please circle YES / NO
   If yes please describe the activity and how long you were exercising for.
Appendix M: Additional information questionnaire: satiety laboratories

ID:

1. Did you consume any alcohol last night?
   a. Please circle YES / NO
   b. If yes how many standard drinks?

2. Did you consume any beverages apart from water or food between 10am and 12pm today?
   a. Please circle YES / NO
   b. If yes what was it and how much? Please provide serving sizes eg medium apple, 1 cup.

3. Did you consume any beverages apart from water or food between 12 and 2pm?
   a. Please circle YES / NO
   b. If yes what was it and how much? Please provide serving sizes eg medium apple, 1 cup.

4. Did you do any exercise apart from walking between 12 and 2pm?
   a. Please circle YES / NO
   b. If yes please describe the activity and how long you were exercising for.

5. Had you seen Fat v Carbs by Jamie Owen before today?
   a. Please circle YES / NO
   b. If yes approximately how many months ago?

6. Had you seen Fat vs Sugar by the van Tulleken brothers before today?
   a. Please circle YES / NO
b. If yes approximately how many months ago?

7. Do you know which sugar was in your drink today? (please circle one)
   a. Sucrose / isomaltulose / don’t know