The effects of apples and apple juice on acute plasma uric acid production and satiety: a randomised controlled trial

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Abstract

**Background:** It has been suggested that fructose from natural sources including fruit and fruit juice behaves differently in the body than added fructose from processed foods. Furthermore, there is inconclusive evidence regarding whether whole fruit and fruit juice differ in their ability to satiate, and their effects on appetite post consumption.

**Methods:** We conducted a randomised controlled crossover trial, with 64 healthy young adults. The three arms of the study included the consumption of whole Royal Gala apples, 100 % New Zealand apple juice, or a control beverage of glucose or fructose in solution. Mean change in acute uric acid concentration and incremental area under the curve were measured at baseline, and then 30 and 60 minutes post food consumption. Satiety ratings from apple and apple juice were measured using visual analogue scales at baseline, 30 and 60 minutes post consumption.

**Results:** We observed a significant difference in plasma uric acid concentration for all three foods at 30-60 minutes, except the glucose monosaccharide beverage. No significant difference existed between the uric acid increase from apples ($P=0.286$) or apple juice ($P=0.132$) and the fructose beverage. However, the apple juice and fructose beverages displayed a greater rate of increase than whole apples. Some measures of appetite were significantly different between the solid and liquid foods during the first 30 minutes, with whole apple more satiating than apple juice.

**Conclusions:** Fructose control, whole apple and apple juice displayed the same plasma uric acid raising potential. These findings suggest the body treats fructose as fructose, independent of the food source. Whole apples were more satiating than apple juice, providing evidence for the consumption of whole fruit to be encouraged over fruit juice.

Key words: fructose, uric acid, satiety, apples, apple juice, fruit, intrinsic, natural.
Preface

The candidate developed the research protocols together with her supervisor Dr Bernard Venn, co-supervisor Ph.D. candidate Mr Andrew Reynolds and Master of Dietetics candidate Emma Carran.

The effect of fructose from apples, commercially available apple juice and control beverages on plasma uric acid concentration and satiety are presented in this thesis. These data are a component of a larger set, which includes acute plasma uric acid response to sugar-sweetened beverages, changes to blood pressure, and gastrointestinal symptoms experienced on consumption of the intervention foods. Genotyping will be undertaken on DNA collected during the study, which is not a component of this thesis. Master of Dietetics candidate Emma Carran will present data from a commercially available, sugar-sweetened beverage separately.

In partnership with Emma Carran, the candidate shared the responsibility of recruitment, dietary instruction, procurement of test foods, food preparation, participant attendance, data collection and laboratory analysis. The candidate was primarily responsible for the design of the satiety and gastrointestinal questionnaires, for data entry, and collating the results.

In order to standardise portion size, the fructose content and sugar profiles of the test foods (Royal Gala apples, and commercial 100 % apple juice) were analysed by Cawthron Institute Laboratories Ltd, Nelson, New Zealand. All other analyses were carried out by the candidates in the Department of Human Nutrition, University of Otago, Dunedin, New Zealand.
Acknowledgements

Firstly to my supervisor **Dr Bernard Venn**, thank you for your guidance and wisdom, and for giving us the opportunity to conduct a randomised controlled trial.

To Ph.D. candidate **Andrew Reynolds** for your dedication, you went beyond your obligations to this project. Our research experience would not have been the same without your input.

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To **Keiran Columb** and **Ivy Salih**, for aiding us during the design phase and data collection phases of our trial. Your experience was indispensable. To the other staff who aided us during data collection (**Angel** and **Marlene**), thank you for coming on board and helping us despite the early morning starts.

To our **participants**, thank you for your dedication to attending the test sessions, we could not have conducted this project without you.

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To **Mum, Dad** and **Nana**, thank you for your support, both emotional and financial throughout this process. It has been a long five years. It was your genes that allowed me to persevere and succeed throughout my time at university.

Thanks to my **flatmates** for your great cooking, and to **Kurt** for your constant support.

Finally, to the ‘**Sisterhood**’, I would not trade you for anything. Our days spent in the office together will be some of my fondest memories from university. I look forward to working with you in the future.
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## Abbreviations

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<tbody>
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<td>ANS</td>
<td>Adult Nutrition Survey</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>HFCS</td>
<td>High fructose corn syrup</td>
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<tr>
<td>iAUC</td>
<td>Incremental area under the curve</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>L</td>
<td>Litre</td>
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<td>mg</td>
<td>Milligram</td>
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<td>µg</td>
<td>Microgram</td>
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<tr>
<td>µmol</td>
<td>Micromole</td>
</tr>
<tr>
<td>n</td>
<td>Number of participants</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<td>VAS</td>
<td>Visual analogue scales</td>
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Introduction

It has been suggested the free fructose present in fruit does not exert the same adverse effects on blood uric acid production and subsequent gout risk as fructose added to processed food in the form of sucrose or high fructose corn syrup (HFCS) (1-3), although there is a popular view that free fructose behaves the same as added fructose within the body therefore should be avoided. Extensive research into the effects of a high fructose diet, particularly the effect of HFCS use in the US has observed an association with metabolic diseases such as gout (3-5). To date, there are limited trials investigating the effects of free fructose from natural food sources, including fruit and fruit juice at ‘realistic’ levels, and subsequent uric acid production. Inconclusive results from testing excessive doses of fructose on uric acid concentration and satiety have arisen from previous work with few participants, or from prospective cohort studies (3, 4, 6-8).

Prospective cohort studies have observed an association between added fructose and the risk of disease associated with the metabolic syndrome (gout, cardiovascular disease and diabetes) (3, 4, 9, 10). The aforementioned diseases are highly relevant in Western society and to New Zealand (11-14). Previous studies have observed that uric acid increases with fructose doses exceeding 60-80 g per day from fruit (15, 16). Other studies have examined the physiological response to fructose in amounts well above typical intake (200 g) (6-8). In New Zealand, it was determined adults consume approximately 39-49g fructose per day (17), with fruit contributing 18 % to the total sugar intake of the adult New Zealand diet (17), which is a lower amount than commonly tested doses of fructose (18). Less than 100 g per day has been suggested to have minimal effects and might instead be beneficial (18).

Given that fruits contain fructose, which has perceived negative effects but are recommended as part of a balanced diet (2), it is important to determine if the fructose from fruit has negative health outcomes. Whole apples have a high free fructose to sucrose ratio when
compared to other fruits (19) and are the second most popular fruit consumed by New Zealanders, after bananas (20). It is also important to consider fruit as a whole food, instead of focussing solely on fructose content.

Fruit juice is considered by some as a greater source of nutrients present in fruit and a more convenient means of consuming these nutrients (21). Liquid foods have also been reported to result in greater blood glucose and insulin response (22, 23) and be less satiating than whole foods, such as whole fruit (23-25). Mechanisms by which whole fruit may be considered more satiating include the presence of fibre, requiring greater mastication and inducing gastric distension during the cephalic phase, suspending the hunger response (26). Fibre may also delay absorption of nutrients in the small intestine (26).

The consumption of natural fructose sources (fruit and fruit juice with no added sweetener) has not been tested in realistic amounts in a randomised controlled trial. Consequently, it is not known to what extent this amount of fructose from these sources will raise the concentration of uric acid, if at all. It is important to determine the effect of a realistic fructose dose, as it is likely results studies investigating excessive fructose consumption have little relevance to public health policy, as daily fructose consumption by the population is likely to be less (18). Increasing our understanding on this topic may allow for more targeted advice in the prevention and management of gout and diseases associated with hyperuricemia.

The consideration of uric acid response to apples and apple juice enabled data collection on the second objective of this research, satiety from the consumption of apples versus apple juice. Satiety is of interest, as the satiating properties of whole fruit in comparison to fruit juices (with no added sugar) is further evidence to promote the consumption of whole fruit rather than fruit alternatives. Increased satiation from the consumption of whole fruit if observed, provides a logical limiting factor to the amount of fructose consumed from natural
sources in any one sitting. This randomised controlled crossover trial was designed to assess the acute uric acid response to realistic amounts of fructose from apples and apple juice in comparison with free-monosaccharide beverages. In addition, we aimed to document any differences in satiety between whole apple and apple juice, as a secondary outcome, given that both foods provide more to the diet than just fructose.
Literature Review

1.1 Methodology of literature review

The literature gathered for this review was identified using the online databases: Medline via Ovid, Science Direct and Pubmed. Keywords used in searches included ‘fructose’, ‘uric acid’, ‘apples’ ‘fruit’ and ‘satiety’. The references of articles identified in our online search were inspected for further articles not yet captured within our search criteria. Relevant trials were randomised and controlled with human participants.

1.2 Introduction

Fructose has a marred reputation largely due to its growing prevalence in the westernised diet, in line with the population’s increasing body mass index (BMI) and rate of non-communicable diseases. The over consumption of fructose from sugary beverages and processed foods corresponds to the increasing prevalence of metabolic syndrome. It is branded as a leading contributor to the Western obesity epidemic and related diseases, of which there is no stand-alone cause. Fructose consumption has also been linked with hyperuricemia, a risk factor for the incidence of gout (2, 3, 27, 28). Whether the source is high fructose corn syrup (HFCS) or the more prevalent sucrose in the New Zealand diet, the presence of fructose in processed food is likely contributing to rising obesity rates (5, 29).

A correlation between gout, and chronically high uric acid levels (hyperuricemia), and fructose has been observed in prospective studies after the consumption of substantial amounts of added fructose, but not with fruit consumption (3, 4). Habitual fructose consumption coupled with genetic susceptibility is suggested to be a contributing factor to the increasing prevalence of gout in the population (30, 31), particularly in those of Māori and Pacific Island ancestry (13, 30). Hyperuricemia has been linked to symptoms of the metabolic syndrome, including overweight and obesity (4, 5, 27). During 2006, it cost the New Zealand
government over 623.9 million, or 4.4% of total health care expenditure (15.4 billion) to treat obesity-related conditions (32), to which hyperuricemia contributes.

This review considers the issues surrounding an increase in intake of fructose consumption with gout and elevated uric acid, as health outcomes and the respective relationship with fructose from fruit and fruit juice. Popular media sites, widely read by the public, often portray fructose in a negative light (33). Perceptions of the public may be that fruits provide unnecessary calories or sugar, without recognising a valuable source of vitamins and minerals, or other proven health benefits (2, 34-37).

Popular diets and some researchers often recommend minimising fruit consumption, as they are well-known sources of fructose, which has been labelled a ‘bad sugar’ (4, 38, 39). The rationale behind these claims includes the potential to cause fat deposition, irregular eating patterns or interference with satiation (39-41). These claims may cause confusion amongst the public; given New Zealand public health strategies encourage people to include adequate servings of fruit and vegetables into their diet (42, 43). Individuals may choose to restrict or avoid fruit as recommended by some health promoting websites (44). As society continues to look for easy strategies for weight loss, these conflicting messages may become detrimental to the credibility of the advice given by health professionals.

1.3 Fructose structure

Fructose is a six-carbon chain or ‘hexose’, and ‘ketose’ due to the ketone group present at C2 (45).

\[
\begin{align*}
1 & \text{CH}_2\text{OH} \\
2 & \text{C} = \text{O} \\
\text{HO} & - 3 \text{C} - \text{H} \\
\text{H} & - 4 \text{C} - \text{OH} \\
\text{H} & - 5 \text{C} - \text{OH} \\
\text{H} & - 6 \text{CH}_2\text{OH} \\
\end{align*}
\]

Figure 1. structure of D-fructose in linear form (45)
1.4 Fructose Absorption

1.4.1 Absorption in the small intestine

Fructose is absorbed from the small intestine through facilitated diffusion (46) in a similar process to glucose, predominantly utilising the transport proteins Glucose Transporter 5 (GLUT 5) and Glucose Transporter 2 (GLUT 2) (47). While GLUT 5 was traditionally identified as a basal membrane glucose transporter, it has a higher affinity for fructose (46, 47) and has been observed within many cells (48). Glucose Transporter 2, which has a low affinity for fructose, transports the sugar out of the enterocyte, and into liver but to what extent is undetermined (47-50). Glucose transporters 7,8 and 12 may also transport fructose (47). Fructose absorption is higher if glucose is present, when absorbed in the form of sucrose, producing a ‘synergistic effect’ (51). Isotope tracer studies were used to quantify this effect, where it was determined the rate of fructose oxidation increased when ingested with glucose (51). This finding suggests fructose transport may be glucose-dependent (46, 48).

1.5 Fructose Metabolism

Fructose is primarily metabolised in the liver (45). The monosaccharide undergoes phosphorylation through the action of fructokinase (keto hexokinase) and adenosine triphosphate (ATP) (52, 53). Fructose it is phosphorylated at the first carbon forming fructose 1-phosphate (fructose + ATP → fructose 1-phosphate) (52, 53). This process occurs rapidly as fructokinase has a high affinity for fructose (52, 54) and occurs faster than glucose phosphorylation (52).

Secondly, fructose 1-phosphate is cleaved by aldolase B (fructose 1-phosphate aldolase), to dihydroxyacetone phosphate and glyceraldehyde, which are glycolysis intermediates (54). To enter the glycolysis pathway both intermediates must undergo further phosphorylation (54). Dihydroxyacetone phosphate is phosphorylated to glyceraldehyde 3-phosphate by the enzyme
triose phosphate isomerase (Figure 2) (55). Glyceraldehyde is also converted to glyceraldehyde 3-phosphate through phosphorylation by triose kinase and ATP (Figure 2) (55). These units enter the gluconeogenesis pathway to form glucose (46).

This process is possible in multiple tissues, as fructokinase is observed in the liver, intestine and kidneys (46). Hexokinase, the equivalent fructose enzyme is present in muscle cells, allowing fructose metabolism to proceed in many tissues if ingested fructose concentrations are high (45) (Figure 2). In muscle, hexokinase phosphorylates fructose at position C-6 forming fructose 6-phosphate, which is an intermediate of glycolysis and can enter into the pathway, requiring no further modification (55).

Figure 2. Pathways of fructose metabolism in the muscle (left), and in the liver (right) (45)
1.6 Uric acid production

Previous work has determined the ingestion of fructose can result in the production of uric acid (3, 4), which in high concentrations is classified as hyperuricemia. Excess uric acid formation occurs with the catabolism of adenine nucleotides ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP), (AMP + ATP ↔ 2 ADP) (53, 54, 56). This process was determined with a 50 g intravenous infusion of fructose (53). Due to the rapid phosphorylation of fructose, which depletes ATP and Pi, metabolism cannot continue (54). A depletion of ATP and organic phosphate (Pi) leads to the degradation of AMP (53). Adenosine triphosphate and Pi inhibit deaminase and 5’-nucleotidase; consequently, AMP is converted to inosine monophosphate (IMP) when inhibition is no longer present (53, 54).

Inosine monophosphate is converted to inosine by the dephosphorylation by 5’-nucleotidase (54). Inosine is the precursor of uric acid, which is phosphorylated to hypoxanthine, then oxidised to xanthine (45). Xanthine is further oxidised to uric acid (45). As humans do not possess the enzyme uricase, uric acid is the end product of purine metabolism and is excreted via the urine (45, 57, 58). The absence of uricase increases the likelihood of the development of hyperuricemia (54, 57, 58). Nonetheless, not all people with hyperuricemia progress to gout (59, 60), suggesting there are other genetic mechanisms involved (31).

Studies investigating antioxidant status after fruit consumption observed an increase in uric acid concentration after consuming fruit juice (16) or fruit (15). Thirty-six grams of fructose from apples induced a modest but non-significant, transient rise in plasma urate concentration. Sixty-four grams of fructose in the form of 1000 g apples and a fructose beverage induced a 31 µM (µmol/L) rise in urate concentration. No significant difference was observed between the food sources of fructose (15). However, this study did not include the sucrose concentration of the apples in the total fructose calculation, potentially underestimating the concentration of fructose (15). In a longer term study conducted in the 1950’s it was
determined there were no detrimental effects of eating a fruit diet supplemented with nuts and avocado for a prolonged period (61).

Figure 3. The relationship between fructose metabolism and the formation of uric acid in the liver. P: phosphate, AMP: adenosine monophosphate, ADP: adenosine diphosphate, ATP: adenosine triphosphate, Pi: organic phosphate, GMP: guanosine monophosphate, PRPP, 5-phosphoribosyl-1-pyrophosphate, IMP: inosine monophosphate (54)

1.7 Sources of fructose

1.7.1 Intrinsic

Fructose exists intrinsically in foods as a free monosaccharide, termed ‘free fructose’ or bound to glucose in a disaccharide as sucrose (55). Glucose and fructose are bonded through a glucosidic alpha 1-2 bond, at C1 and C2 respectively (45) (Figure 4). Fructose is present in processed foods but also naturally occurs in food such as fruit, honey or some root vegetables (62). As a result, a diet containing minimal added sugar but including fruit contributes fructose.
Added sugar intake and the effects of a fructose load

Apple juice intake and the effects of a fructose load on subsequent uric acid levels are of interest in this thesis. Fruit juice is different to fruit drinks, which contain concentrate and added sugar rather than pure fruit juice (21). This thesis will investigate 100 % fruit juice.

**1.7.2 Fruit juices**

Processed forms of fruit i.e. natural fruit juices compile other sources of fructose and include apple juice. Around 60% of the calories available in apple juice may be from fructose (5). Apple juice intake and the effects of a fructose load on subsequent uric acid levels are of interest in this thesis. Fruit juice is different to fruit drinks, which contain concentrate and added sugar rather than pure fruit juice (21). This thesis will investigate 100 % fruit juice.

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**Figure 4.** Monosaccharides glucose and fructose bonded through a 1-2 glucosidic bond to form sucrose (45).

**Table 1.** Sugar composition of some common fructose containing foods (g/100g (19, 63)

<table>
<thead>
<tr>
<th>Fructose containing foods</th>
<th>Total Fructose (g)</th>
<th>Sucrose (g)</th>
<th>Glucose (g)</th>
<th>Total Sugars (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple raw, with skin*</td>
<td>5.90</td>
<td>1.86</td>
<td>2.71</td>
<td>10.48</td>
</tr>
<tr>
<td>Banana, raw*</td>
<td>4.85</td>
<td>2.39</td>
<td>4.98</td>
<td>12.23</td>
</tr>
<tr>
<td>Fruit juice, apple*</td>
<td>5.73</td>
<td>1.26</td>
<td>2.63</td>
<td>9.62</td>
</tr>
<tr>
<td>Carbonated beverage, sprite (HFCS sweetened) *</td>
<td>6.25</td>
<td>0.00</td>
<td>4.14</td>
<td>10.42</td>
</tr>
<tr>
<td>Carbonated beverage, sprite*</td>
<td>5.19</td>
<td>0.65</td>
<td>3.13</td>
<td>8.98</td>
</tr>
</tbody>
</table>

* (19), * (63)
1.7.3 **Added sugars**

Added sugars or free sugars from extrinsic sources are added to food during production and contribute to fructose intake in the form of sucrose, free fructose or HFCS (17, 36) are added to food during production and contribute to fructose intake (62, 64).

1.7.4 **High fructose corn syrup**

A large proportion of fructose intake is provided by foods containing HFCS, which has replaced the use of sweeteners from sugar cane and beets (62). The USA is the primary consumer of HFCS, but its use as a sweetener in foods is increasing worldwide (65). High fructose corn syrup is formed through the hydrolisation of corn starch, which converts glucose molecules into fructose (62). High fructose corn syrup is a ratio of fructose to glucose monosaccharides (62, 64). High fructose 55 is a ratio of 55:45 (fructose: glucose) and is the most commonly used sweetener in soft drinks and sweetened beverages in the US (62, 64).

1.7.5 **Sugar sweetened beverages**

Sugary beverages appear to be the primary contributor of excess fructose and free sugar in the population (17, 66). Excess sugar consumption is speculated to contribute to an increase in BMI and prevalence of Type 2 Diabetes Mellitus (T2DM), cardiovascular disease (CVD), hypertension (HTN), metabolic syndrome, non-alcoholic fatty liver disease and gout within the New Zealand population (4, 10, 12, 28, 67-69). The New Zealand Beverage Guidance Panel aims to minimise the consumption of sugar-sweetened beverages, to aid New Zealand to become sugary beverage free by 2025 to minimise the risk of related diseases (70, 71).

1.8 **Sugars and fructose consumption in New Zealand**

Sucrose is the primary added sugar during food manufacture in Australia and New Zealand (17). Although sugar-sweetened beverages in New Zealand do not contain HFCS, a correlation between gout prevalence and the habitual consumption of sugar-sweetened
beverages has been observed here (69). The total fructose amount consumed from the New Zealand diet can be estimated using results from the Adult Nutritional Survey (ANS) 2008/09 (17), by adding free fructose to half of the median usual daily intake of sucrose presented in the ANS findings (17). Consequently, total median intake of fructose for males and females was determined to be 49.1g and 39.3g respectively (17). While this observational data can describe the current intake, the minimum level of fructose tolerated by the liver before adverse effects occur is still largely unknown. There is limited research investigating realistic habitual fructose consumption and gout risk.

The Ministry of Health predicts that 29% of carbohydrate consumed within the New Zealand adult diet is comprised of free sugars in the form of non-alcoholic sugary beverages (43). This is a problem due to the high energy density, low nutrient value and the aforementioned negative health effects associated with the consumption of sugary beverages (72).

In New Zealand popular sugar sweetened beverages including sugary beverages (carbonated, energy or sports drinks) contain approximately 9.9-10.2 g of total sugar per 100ml, and can contribute approximately 500 kilojoules (kJ) to a person’s daily energy intake with just one glass (73). Half is presumed to be fructose if the product is sweetened with sucrose, or a higher proportion if sweetened with HFCS.

1.9 Fructose and health

1.9.1 Perceived health benefits of fructose

Fructose was previously recommended as an alternative sweetener for non-insulin dependent diabetic patients (51, 54), as it is claimed to not induce a postprandial insulin response (51) and to significantly improve glycaemic control in non-insulin dependent diabetic patients (74). The production of uric acid from fructose consumption has known pathogenic effects.
An increase in antioxidant status of participants ingesting fructose has been determined and attributed to an increase in serum uric acid concentration (7, 8, 15, 16, 75).

1.9.2 Fructose health claim

The reported reduction of post-prandial glycaemic response prompted the European Food Safety Authority (EFSA) to provide guidelines for fructose use in food and beverages (76). The resulting policy allows a health claim to highlight the health benefits of fructose on the front of foods and beverages containing the sugar (76). The EFSA panel states “consumption of fructose leads to a lower blood glucose rise than the consumption of sucrose or glucose,” (76) despite EFSA’s acknowledgement that over-consumption of fructose may lead to the development of metabolic abnormalities (76). In addition, EFSA stated there was no evidence to suggest an upper intake limit of fructose intake (77). This policy may allow the food industry to direct its marketing efforts to those individuals aiming to reduce their blood glucose levels.

1.10 Diseases associated with excess fructose consumption

1.10.1 Gout

Hyperuricemia is the primary risk factor for gout and is the primary disease associated with fructose consumption (3). It occurs when serum urate levels induced by diet rise above the saturation concentration in body fluids (approximately 0.4mmol/L (400 µmol/L)) (78). A single dose of fructose may acutely elevate uric acid, but the habitual intake of fructose and the related physiological processes is suggested to determine chronically saturated uric acid levels (hyperuricemia) and possibly gout (4). Gout is characterised by the crystallisation of mono sodium urate at saturation levels in the joints (31, 79), which causes painful episodes or ‘attacks’ of symptoms similar to that of arthritis (78). ‘Gout attacks’ occur in the peripheral joints or extremities, with severe pain, swelling, and inflammation (60). Without treatment, gout can permanently damage the joint and kidney tissues (79). When combined with other
risk factors, such as hypertension and diabetes, hyperuricemia may increase the risk of heart disease and kidney failure (60, 80). Those with a family history of gout were observed to be more susceptible to the uric acid increasing mechanism of fructose and gout development, compared to those without a family history of gout (81).

**Worldwide prevalence of gout**

The increasing prevalence of gout is not only confined to New Zealand, but has occurred worldwide particularly in Western countries (82, 83). Given the detrimental nature of gout as a disease, further research is required into the causality of specific risk factors in Western society. The number of countries adopting a westernised diet makes this a pertinent issue. Gout is predominantly genetic, although environment or diet is said to play a part, as ‘trigger foods’ can induce a gout ‘attack’. Foods that commonly cause gout attacks contain purines; present within the genetic fraction of food including meat and seafood (84). Purines are the basic structure of DNA, which when metabolised within the human body release uric acid into the bloodstream (45).

**Gout prevalence in New Zealand**

The crude prevalence of gout is 2.69% in New Zealand, which is one of the highest rates worldwide (13, 14, 85). Gout is most common in older men and postmenopausal women (14, 86) (87, 88), particularly those of Pacific, Māori, or New Zealand European ancestry in descending order (13, 14). It is predicted approximately 25% of elderly men (>65 years) of Māori and Pacific peoples are at risk of gout (13). This number may be increasing when compared to the prevalence of gout in 1978 (89).

Genetic research suggests the difference in urate response between ethnicities in New Zealand is likely due to variants in the gene *SLC2A9*, which controls the expression of the GLUT 9 transporter (*SLC2A9*). GLUT 9 is observed in the renal tubule and is responsible for reabsorbing glucose and fructose (90). *SLC2A9* was determined to be responsible for urate
reabsorption, with many other urate transporters, at the same site along (31, 90). Genetic variants of this gene may result in an increase or decrease in the rate of uric acid excretion (90). Māori and Pacific peoples are speculated to be at risk of gout because they are genetically susceptible to hyperuricemia (13, 14, 31).

1.10.2 Obesity

Unlike glucose, fructose consumption does not induce an insulin response (51); therefore, it is speculated fructose has no mechanism by which satiety is regulated post prandial ((41, 91). Thus, the interaction between the hormones insulin and leptin, which together regulate food intake and body weight, suggests an underlying relationship between fructose and weight gain (41). Previous trials suggest that large doses of glucose and fructose both induce weight gain in participants (92). It is inconclusive whether fructose is more obesogenic than glucose.

1.11 Satiety and fructose

1.11.1 Solid versus liquid foods and satiety

It has been determined it is relatively easy to consume large amounts of energy through the consumption of sugary beverages. Over-consumption may occur through the lack of compensation for calories ingested during successive meals (93, 94). It was observed that participants consumed greater energy when a beverage was consumed with solid foods (25, 93, 95), suggesting sugary beverages do not induce a strong satiety response (25, 94-96).

It is suggested by the World Health Organization (WHO) (97), that excess energy intake from sugary beverages is promoted by the lack of fibre, protein and fat, which leads to reduced satiation (36). The evidence surrounding satiety after liquid versus solid carbohydrate is conflicting and inconclusive (91, 98). Although there is persuasive evidence sugary beverages are easy to consume in large amounts compared to solid foods (98).
There is evidence that solid fruit is more satiating when compared to fruit juice (25), which may provide explanation for why the consumption of sugar-sweetened beverages is associated with gout (4, 69). It was observed the ease of consumption was greater when drinking fruit juice than whole fruit (25). Physiological factors other than the nutrient content of a beverage are said to contribute to a lack of satiation (99). Lack of mastication (100), and decreased gastrointestinal transit time, due to the lack of pectin and fructan fibres usually present in fruits, may contribute (101, 102). The resulting lack of satiation may contribute to the increased consumption of beverages (99, 103, 104) and induce weight gain compared to the consumption of whole fruit matched to the sugar content (25).

1.12 Rationale for our research

There is limited work considering the effects of fruit and fruit juice consumption regarding gout risk. Fructose is known to increase plasma uric acid, but limited studies test foods that are natural sources of fructose. Our randomised controlled crossover trial is novel, as the consumption of realistic servings of natural fructose and its effect on uric acid concentration has not been investigated in New Zealand. Due to New Zealand’s high prevalence of gout, more information needs to be collected to eliminate the fear of fructose consumption from fruit. The magnitude of acute uric acid production after consumption of fruit and fruit juice in amounts included in the Ministry of Health Dietary Guidelines needs to be investigated (42, 43).
Objective statement

*Aim:* To determine the effects of the consumption of Royal Gala apples and commercially available 100% apple juice (with no added sugar) on acute uric acid production, and satiety in a cohort of healthy young adults. The intervention includes a “small” and “large” serving of apples (205 and 410 grams) and apple juice (170 and 340 millilitres). A second and separate objective was to measure participant satiety using visual analogue scales (VAS).

*Objective one:* To test whether the consumption of natural sources of fructose from common fruit and fruit products, in realistic serving sizes, has a significant effect on acute blood uric acid concentration, when compared to a pure fructose beverage.

*Objective two:* To examine the differences between the satiating properties of whole apples versus 100% apple juice with no added sugar, using VAS satiety measures.
Methods

A randomised controlled crossover trial, which ran over four weeks was undertaken at the Department of Human Nutrition, University of Otago, Dunedin, New Zealand, between February and March 2015.

1.13 Ethical approval

The University of Otago Human Ethics Committee (Health) approved this study in November 2014 (Ethics Committee reference number 14/204) (Appendix A, B). The trial was registered with the Australian New Zealand Clinical Trial Registry (ANZCTR:12615000215527).

1.14 Enrollement and participation requirements

Participants were a convenience sample of University of Otago Human Nutrition students, recruited in February 2015. Participants were provided a full study information sheet and consent form anytime online before and during the study period. Participants were required to provide informed consent before entering the study. Participants were required to be between 18 and 65 years of age, and to have normal glucose tolerance, defined by the World Health Organization as a fasting blood glucose concentration below 5.6 mmol/L (105).

Exclusion criteria were diabetes mellitus, cancer, a digestive condition that may affect the absorption of fructose, a recent stroke, and women who were pregnant. A demographic questionnaire was used to screen participants for study eligibility (Appendix A). Participation involved consumption of an allocated intervention food, providing blood samples for plasma uric acid and glucose analysis, and completion of several questionnaires. Ethnographic and lifestyle data were collected. A satiety questionnaire was completed at baseline and 30 and 60 minutes following consumption of the test foods. Blood pressure, height, and weight were measured at baseline.
1.15 Randomisation

Seventy-three participants were invited to participate and then randomised into the study groups (58 females, 15 males) Figure 1. Participants were block randomised by sex into one of the intervention groups to allocate evenly males and females into the three intervention arms. For each group, participants were randomised to order and test sessions. Within each test session, participants were randomised to staggered attendance times. These times were often not adhered to in practice. Randomisation was undertaken by Dr Jill Haszard with STATA (STATA/1C version 13.1) computer software.
Figure 5. Consort Diagram: Flow of participants through the study, in control, apple juice and apple groups.
1.16 Intervention foods

Apples and apple juice were selected as intervention foods due to their natural fructose content, and because they are widely available and accepted foods. Compared to other fruit, apples have a high fructose content, of approximately 5.9 grams per 100 grams (19) (Table 1). The serving sizes provided within the study were designed to represent the normal consumption of fruit and fruit juice, to give the study a practical application. Intervention foods were purchased from a local supermarket from late February to late March 2015.

Royal Gala apples were chosen as they were in season during the study period. A ‘Mill Orchard’ brand of apple juice was chosen, as it contained 100 % apple juice made from New Zealand grown apples. The apple juice had no added sugar, preservatives, colours, flavours or concentrate (106). The variety of apples within the juice could not be determined as the juice is made from a blend of apple varieties (106).

1.17 Control foods

The control group received a fructose or glucose monosaccharide beverage (4.45 % concentration) dissolved in 600 mL of soda water, in random order. A fructose beverage was used as a positive control as the fructose monosaccharide is readily absorbed without enzymatic degradation (107), or interference from fruit fibres and phytochemicals. A glucose beverage was used as a negative control because no rise in plasma uric acid concentration was expected, as there is no known pathway by which glucose is converted to uric acid (53, 108).

1.18 Sugar composition analysis

Cawthron Laboratories Ltd, Nelson, New Zealand, assessed the free fructose, sucrose and glucose content of the intervention foods. Analysis occurred during February 2015. The sugar content of the foods was quantified to ensure consistency across the study. The fructose
amounts of apple and apple juice were matched to the fructose amount provided to participants in the control beverages (26.7 grams).

Total fructose was calculated by adding the free fructose and half of the sucrose amount for each intervention food. The fructose concentrations of the small servings were set at half the fructose concentrations in the large servings. By matching the fructose content of the control and large intervention servings, we could directly compare the effects of intervention foods against the control beverages.

Cawthron Laboratories Ltd conducted a second analysis during March 2015 on samples of apples and juice consumed during the trial, to ensure the amounts of fructose were comparable among intervention foods. Little variation existed between the two samples. Free fructose, glucose and sucrose concentrations per 100 g varied by 0.3g, 0.4g, 0.2g respectively for Royal Gala apples, and 0g, 0.8g, 0.3g respectively for apple juice. Results from the food composition analyses are presented in Table 2 and are attached as appendices (Appendix C).

Table 2. Results from Cawthron Laboratories Ltd sugar assessment, February 2015

<table>
<thead>
<tr>
<th></th>
<th>Apple (n=23)</th>
<th>Apple Juice (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fructose (g/100g)</td>
<td>5.20</td>
<td>7.40</td>
</tr>
<tr>
<td>Fructose from sucrose (g/100g)</td>
<td>1.30</td>
<td>0.45</td>
</tr>
<tr>
<td>Total fructose available (g/100g)</td>
<td>6.50</td>
<td>7.85</td>
</tr>
<tr>
<td>Amount of intervention food given containing 26.7 g fructose</td>
<td>410g</td>
<td>340mL</td>
</tr>
</tbody>
</table>

1.19 Amounts administered

The apple and apple juice intervention groups consumed a small (13.4 grams) serving and a large (26.7 grams) serving of fructose in pre-randomised order. Intervention food servings are presented in Table 3. The apple group received chopped, and unpeeled whole Royal Gala
apples with the stalk, core and seeds removed. Apples were stored in the fridge before the test session and removed the morning of the test session. The apple pieces were covered with lemon juice to prevent browning. All foods were weighed using Sartorius Auto Cal Scales (AG:CP424025; Germany).

Table 3. Large servings of intervention foods containing 26.7 grams of fructose and small servings containing 13.4 grams

<table>
<thead>
<tr>
<th></th>
<th>Apples (Royal Gala)</th>
<th>Apple Juice</th>
<th>Control (fructose/glucose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small serving</td>
<td>205 g</td>
<td>170 mL</td>
<td>-</td>
</tr>
<tr>
<td>Large serving</td>
<td>410 g</td>
<td>340 mL</td>
<td>600 mL</td>
</tr>
</tbody>
</table>

1.20 Intervention

The acute effects on circulating uric acid concentration and satiety after consumption of the test foods (Royal Gala apples and 100 % apple juice) in comparison with the control beverages (glucose and fructose monosaccharides) were examined. Participants presented at the test facility twice over a four-week period and were required to fast from 10 pm the night before.

Participants were instructed to avoid alcohol and to eat a meal rich in carbohydrates the previous night, prior to their allocated study time. Participants were also asked to avoid vigorous physical activity; subsequently, it was recommended participants drove or walked slowly to the testing facility, then rested, before commencing the study. To survey compliance with pre-test instructions, participants were required to fill in a questionnaire regarding their alcohol intake, food consumption, and level of physical activity during the 24 hours prior to the test session, to minimise confounding (Appendix F). Approximately 96 % of participants
were compliant with instructions. The remaining participant’s data were monitored with no outliers observed. No adjustment was made for outliers.

Participants were then administered the randomly allocated intervention food, which was to be consumed within 10 minutes. During the second visit, participants received the same test food, but a different quantity (large or small serving). Order of intervention was randomised.
Figure 6. Study design: Participants were randomly allocated to apple, apple juice or control groups, and an attendance week (weeks one and three or two and four).
1.20.1 Outcome measures

**Body Mass Index**

Research assistants trained in anthropometric measurement (ISAK level one accreditation), recorded participant heights using a calibrated Holtain Limited Stadiometer. Body weight was measured using calibrated Seca Alpha scales (model 770). Participants had removed shoes and socks before weight and height measurements were taken. The data were used to calculate body mass index (BMI), for each participant using the equation: weight in kilograms/height in metres squared (kg/m²).

**Blood pressure**

Resting baseline blood pressure was taken using an OMRON digital automatic blood pressure monitor (Model Hem-907). The final blood pressure reading was an average of three readings.

**Uric acid and glucose**

After baseline data capture, participants underwent a 90-minute plasma uric acid and glucose response profile on consumption of an apple, apple juice or control beverage. The start time of the experiment (Time 0) was the commencement of the consumption of the intervention food. Wheat bags were provided to participants to warm their hands to ease blood collection. Five hundred μL blood samples were obtained from a single fingertip puncture, using BD Microtainer contact activated lancets. Blood samples were collected into BD Microtainers coated with the anticoagulant K2EDTA (**Appendix E**).

Blood glucose was measured over the test period to capture acute changes, induced by the intervention foods. Any abnormal glucose concentrations were re-tested. Blood glucose concentrations were monitored to ensure participants were of normal glucose tolerance (105).
**Satiety**

Visual analogue scales (VAS) were used to assess hunger, satisfaction, fullness, and prospective food consumption ratings induced by the intervention foods (Appendix G). The participants completed four VAS, developed and validated by Flint et al. (109) at baseline, then 30 and 60 minutes after consumption.

The VAS scales were 100 mm long with extreme ratings located at either end. The scales assessed hunger ($0 = I$ have never been more hungry, $100 = I$ am not hungry at all), satisfaction ($0 = I$ cannot eat another bite, $100 = I$ am completely empty), fullness ($0 = Not full at all, 100 = totally full$), prospective food consumption ($0 = nothing at all, 100 = a lot$), comfort ($0 = very uncomfortable, 100 = very comfortable$). A VAS for comfort level was added to the questionnaire as it was predicted participants might feel uncomfortable after consuming the large intervention servings within 10 minutes. The scales were quantified with a ruler, providing a value (mm) between 0 and 100.

**Demographic questionnaire**

At each visit participants completed a questionnaire collecting baseline information regarding gender, ethnicity, age, family history of gout, smoking status, medication, supplement use, food allergy/intolerance, diagnosis of chronic disease, disease of the digestive system and pregnancy. Ethnographic data was prioritised according to the Ministry of Health ethnicity data protocol, and participants identifying as multiple ethnicities were only represented once in the table (110)

**1.20.2 Laboratory procedures**

Whole blood glucose concentration was measured using a $5 \mu$L spot of blood with a calibrated HemoCue glucose 201+ unit, at 0, 30 and 60 minutes. The first drop of blood was discarded by wiping it from the fingertip with lint-free gauze to prevent dilution of the blood samples from the sanitising alcohol wipe.
Plasma was recovered from 500 μL sample of whole blood after centrifuging at 2000 RPM for five minutes. The plasma was pipetted off the supernatant and stored at -70 °C for no longer than four weeks until analysis. The analysis was conducted in the Human Nutrition Diabetes and Lipids Laboratories using a Roche Hitachi Cobas c 311 auto-analyser (Roche Diagnostics, Indianapolis, IN, USA). Uric acid concentration was determined by enzymatic colorimetric assay using a UA2 uric acid reagent test kit (Roche Diagnostics), per manufacturer's instructions. A two-point calibration was conducted before analysis and when the UA2 uric acid reagent test kit was changed. Plasma uric acid concentrations were expressed in μmol/L.

Samples were defrosted as required and analysed in 20 sample loads. Machine consistency was determined using manufacturer normal and low controls, and a pooled sample. Pooled samples were included in the assay to measure the between-run and between-day consistency of the assay expressed as a coefficient of variation. The between-run coefficient of variation for the pooled sample, normal and high controls were 2.56%, 2.31%, and 2.72%, at mean concentrations of 260.8 μmol/L, 267.1 μmol/L, and 642.7 μmol/L respectively.

One hundred and twenty microlitres (120 μl) of plasma was the minimum required aliquot for uric acid assay in the Cobas C311 analyser. When too little sample was collected, the available sample was diluted to 80:40, 50:50, 40:80 or a 20:100 ratio of plasma to distilled water. Thirty samples were diluted and corrected using the appropriate dilution factor (8.2% of total data). A dilution curve was calculated before sample analysis and determined to be linear (Appendix H). Therefore, dilution of the samples and the appropriate multiplication factor was taken to be an accurate method to determine the plasma uric acid concentration from low sample volumes.
Successive uric acid concentrations were monitored for error throughout the analysis for each study ID. *A priori*, any samples that were above 410 μmol/L were re-run to check validity, as this concentration had been predetermined as the point at which, crystallisation within the joints might occur in a person with gout. If previous values for that participant were also high, the sample was not re-run. Out of all the samples run, only 1.64% of samples were re-run. None of the re-run samples were determined to be in error, and were within ± 4% of the initial value. Under these circumstances, only the first assay result was used, with the second sample taken as was used as confirmation of an accurate first reading.

1.20.3 Study outcomes

The primary outcomes were change in plasma uric acid in response to the fructose concentration of the intervention foods. A secondary and separate outcome was the satiety response to the intervention foods.

1.20.4 Raw data handling

At recruitment participants were allocated a study identification number, which was used to anonymise participants during the study period. The candidate coded and entered the collected data into 2011 Microsoft® Excel® for Mac (Microsoft Corporation 2011™, United States of America). Excel® was also used to collate and analyse baseline data.

1.21 Statistical methods

1.21.1 Sample size calculation

Results from another study were used to estimate the sample size required for the study (111). Calculations for the sample size used a standard deviation of 80 μmol/L. It was determined 20 participants were required in each test group to detect a 50 μmol/L change in plasma uric acid concentration (111), with 80% power at the significance level of $P < 0.05$. 
1.21.2 Outliers

Outliers were defined as having a successive uric acid concentration increase of greater than 100 \( \mu \text{mol/L} \), and if the value in question was inconsistent with the remaining values for that person, the value was deemed an outlier. Four data points were removed on the basis of being physiologically unlikely. Two pieces of data were replaced with those participant’s baseline data from the second test session.

1.21.3 Analysis

Dr Jill Haszard, a biostatistician within the Human Nutrition Department conducted the statistical analysis in consultation with the Master of Dietetics candidate and study team. 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). The analysis was conducted concerning a pre-established analysis plan.

1.21.4 Plasma uric acid

Plasma uric acid response was assessed through a change in plasma uric acid from baseline at 30 mins and 60 mins. Investigation of plasma uric acid concentration at 30 and 60 minutes postprandial, compared with baseline was undertaken by mixed model regression analysis, with time as a fixed effect and participant as a random effect.

The model was adjusted for the covariates: sex, age, order of intervention (small serving followed by the large serving or vice versa), smoking, alcohol, family history of gout and time of attendance at the test session. Results are presented as mean difference and standard deviation (with 95% confidence intervals) within the intervention groups, and between servings and groups.

A summary measure of the difference in plasma uric acid response between the intervention foods were determined by calculating the incremental area under the curve (iAUC) and are
presented as mean difference iAUC. Incremental area under the curve was calculated geometrically from baseline to 60 minutes by applying the trapezoid rule. The area above baseline was assigned a positive value and the area below baseline was assigned a negative value. The sum of the iAUC was reported.

Incremental area under the curve response was analysed between small and large servings within groups and was investigated by modelling the change in plasma uric acid or iAUC against serving size, with participants as a random effect. Between-group differences were determined by linear regression (adjusting for baseline in analyses of mean change over time).

1.21.5 Satiety

Analysis was undertaken to identify differences between the whole apple and apple juice groups to investigate differences in satiety. The mean difference was calculated in both analyses using regression models adjusted for baseline. Results are presented as mean change within the intervention groups, compared with baseline, and as the mean difference as a comparison measure in satiety.
Results

Sixty-four people were included in the analysis. Participants were between the ages of 19 and 51 years of age. **Figure 1** shows the flow of participants through the study.

1.22 Baseline characteristics of participants

Baseline characteristics of the participants are presented in **Table 4**. Participants were healthy adults typically in their early twenties, although two participants in the apple and apple juice groups were older. Participants were predominantly female, in approximately a 75:25 (female: male) ratio, and of New Zealand European ethnicity. The majority of participants were within a healthy BMI range. The nine participants who reported a family history of gout (14.06 %) were evenly distributed across the groups.

Mean baseline plasma uric acid and glucose concentrations from both testing sessions were averaged to produce a mean baseline concentration for each group. Mean baseline glucose was within the normal range for people with normal fasting glucose (< 4.8 mmol/L) (105).

There appeared to be no differences in mean baseline satiety VAS ratings before participants consumed their allocated intervention or control food.
## Table 4. Demographics of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Royal Gala Apples (n=23)</th>
<th>Apple juice (n=21)</th>
<th>Control (n=20)</th>
<th>Total (n= 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.3 (6.05)</td>
<td>24.0 (7.29)</td>
<td>20.9 (1.69)</td>
<td>22.4 (5.68)</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>14 (60.87)</td>
<td>12 (57.14)</td>
<td>17 (85.00)</td>
<td>43 (67.19)</td>
</tr>
<tr>
<td>Māori</td>
<td>1 (4.35)</td>
<td>2 (9.52)</td>
<td>1 (5.00)</td>
<td>4 (6.25)</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>1 (4.35)</td>
<td>0</td>
<td>0</td>
<td>1 (1.56)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (30.44)</td>
<td>7 (33.33)</td>
<td>2 (10.00)</td>
<td>16 (25.00)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 (3.17)</td>
<td>22.9 (3.65)</td>
<td>22.6 (2.13)</td>
<td>23.0 (3.04)</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>22 (95.7)</td>
<td>19 (86.4)</td>
<td>20 (95.2)</td>
<td>61 (95.3)</td>
</tr>
<tr>
<td>Previous</td>
<td>1 (4.4)</td>
<td>2 (9.1)</td>
<td>0</td>
<td>3 (4.7)</td>
</tr>
<tr>
<td>Current</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Family history of gout (%)</td>
<td>3 (4.7)</td>
<td>4 (6.3)</td>
<td>2 (3.1)</td>
<td>9 (14.1)</td>
</tr>
<tr>
<td>Blood glucose concentration (mmol/L)</td>
<td>4.8 (0.4)</td>
<td>4.6 (0.5)</td>
<td>4.9 (0.6)</td>
<td>4.8 (0.5)</td>
</tr>
<tr>
<td>Plasma uric acid concentration (µmol/L)</td>
<td>277.4 (70.1)</td>
<td>277.8 (65.0)</td>
<td>267.6 (49.1)</td>
<td>274.4 (62.1)</td>
</tr>
<tr>
<td>Blood pressure (mm/Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>107.6 (11.0)</td>
<td>110.1 (10.4)</td>
<td>109.8 (10.0)</td>
<td>109.2 (10.3)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>60.8 (6.8)</td>
<td>62.8 (8.7)</td>
<td>61.2 (5.9)</td>
<td>61.6 (7.2)</td>
</tr>
<tr>
<td>Baseline satiety ratings (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>48 (26)</td>
<td>49 (23)</td>
<td>42 (20)</td>
<td>46 (23)</td>
</tr>
<tr>
<td>Satiation</td>
<td>73 (21)</td>
<td>68 (19)</td>
<td>71 (14)</td>
<td>71 (18)</td>
</tr>
<tr>
<td>Fullness</td>
<td>24 (22)</td>
<td>24 (20)</td>
<td>23 (20)</td>
<td>23 (20)</td>
</tr>
<tr>
<td>Prospective food consumption</td>
<td>66 (23)</td>
<td>66 (18)</td>
<td>66 (15)</td>
<td>66 (19)</td>
</tr>
<tr>
<td>Comfortableness</td>
<td>55 (23)</td>
<td>64 (20)</td>
<td>54 (18)</td>
<td>58 (21)</td>
</tr>
</tbody>
</table>

All participant data were used in baseline data analysis.
All data are presented as mean (SD) unless stated otherwise
Baseline satiety ratings were measured from fasted participants

### 1.23 Acute plasma uric acid response

Plasma uric acid analysis is presented as the mean difference and standard deviation, between baseline to 30 minutes, then 30-60 minutes within intervention groups and control in Table 2.
Incremental area under the curve (iAUC) is presented as the mean iAUC (μmol/L•h). The mean iAUC is a summary measure of the response to fructose between baseline and 60 minutes and between groups (Table 6 and 7).

Table 5. Adjusted * mean difference in plasma uric acid concentration between baseline and 30 minutes, and 30 and 60 minutes of both large and small servings of all test foods

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference at 30 minutes (95 % CI)</th>
<th>P-value</th>
<th>Difference at 60 minutes (95 % CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-5.0 (-10.9, 0.9)</td>
<td>0.09</td>
<td>-8.4 (-15.3, -1.5)</td>
<td>0.018</td>
</tr>
<tr>
<td>Fructose</td>
<td>25.5 (11, 40)</td>
<td>0.01</td>
<td>26.3 (8.6, 44.1)</td>
<td>0.004</td>
</tr>
<tr>
<td>Large Servings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple 410g</td>
<td>18.7 (8.6, 28.8)</td>
<td>&lt;0.001</td>
<td>10.2 (3.3, 17.1)</td>
<td>0.004</td>
</tr>
<tr>
<td>Juice 340mL</td>
<td>16.4 (9.3, 23.5)</td>
<td>&lt;0.001</td>
<td>22.9 (7.3, 38.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Small Servings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple 205g</td>
<td>8.0 (5.7, 10.9)</td>
<td>&lt;0.001</td>
<td>4.8 (0.4, 9.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Juice 170mL</td>
<td>9.4 (4.1, 14.7)</td>
<td>0.001</td>
<td>9.1 (4.5, 13.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Adjusted for order, hour of test, alcohol, smoking, sex and family history of gout.

'P ≤ 0.05 was considered significant.

‘Fasted or not’, was only adjusted for in second analysis.

Apple (n = 23), Apple Juice (n = 21), Control (n = 22).

Large servings = 410 g apple, 340 mL apple juice, 600 mL of fructose/glucose solution.

Small servings = 205 g apple, 170 mL apple juice.

The statistical model for the change in uric acid between baseline, 30 and 60 minutes was run initially without adjustment for covariates. Subsequently, the model was adjusted for the covariates; time, order, alcohol, servings, smoking, family, sex and family history of gout for a more precise estimate of the results. These adjustments had little impact on mean estimates, indicating there was minimal confounding.
For the iAUC analysis, unadjusted models for the difference in iAUC between the large servings of the intervention foods and the fructose control did not comply with assumptions of linearity. When adjusted for covariates, the homogeneity and normality of the residuals improved. As a result, only the adjusted analysis is presented in this analysis.

Significant increases in plasma uric acid were observed for all of the fructose-containing test foods during both time periods. In contrast, glucose significantly decreased uric acid concentration between 30 and 60 minutes. At 60 minutes, uric acid concentration appeared to be maintained in the small serving of apple juice. After 60 minutes, the uric acid concentration appeared to halve after the small apple serving (Table 5).

The rate of increase in uric acid concentration was not tested for, but it was determined by plotting the adjusted mean difference for each time point and foods that fructose rose steeply between baseline and 30 minutes. After 30 minutes the uric acid concentration tended to plateau (Figure 7). Apples and apple juice appeared to increase uric acid more slowly than pure fructose. The consumption of apples appeared to raise uric acid slower and tended to decline towards 60 minutes. Apple juice also appeared to increase uric acid more slowly than fructose and tended to increase towards 60 minutes.

![Figure 7](image_url)

**Figure 7.** Visual comparison of the adjusted mean difference ($\mu$mol/L•h) between large servings of apple (410 g) and apple juice (370 mL) and fructose control between baseline and 30 minutes, and 30 and 60 minutes. All food servings contain 26.7 g of fructose.
1.24 Incremental area under the curve

The adjusted difference in iAUC between the large and small servings administered to participants is presented in Table 6. There was a significant difference in iAUC between the small and large servings in both the apple and apple juice groups, and a significant difference existed between the glucose and fructose control beverages. The mean change in iAUC tended to be lower in the apple and apple juice groups, compared to the control group.

Table 6. Adjusted* difference in iAUC of small and large servings of test foods and beverages

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean iAUC of small serving $^1$ (µmol/L•h)</th>
<th>Mean iAUC of large serving $^2$ (µmol/L•h)</th>
<th>Mean change$^3$ iAUC (µmol/L•h)</th>
<th>95% CI</th>
<th>P-value $^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>311.3</td>
<td>713.5</td>
<td>412.4</td>
<td>18.4, 806.5</td>
<td>0.040</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>419.6</td>
<td>823.9</td>
<td>426.8</td>
<td>56.1, 795.4</td>
<td>0.024</td>
</tr>
<tr>
<td>Control</td>
<td>-320.9</td>
<td>1174.2</td>
<td>1349.7</td>
<td>750.0, 1949.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Adjusted for order, timing, alcohol, smoking, family history of gout, and sex.
$^+$ P ≤ 0.05 was considered significant.
$^1$ The mean iAUC of the small servings = 205 g apple, 170 mL of apple juice, and the glucose control beverage.
$^2$ The mean iAUC of the large servings = 410 g apple, 340 mL of apple juice, and the fructose control beverage.
$^3$ Mean change of iAUC between the small and large servings of each intervention group (apple = 205 g vs 410 g, apple juice = 170 mL vs 340 mL) or control (glucose vs fructose).

A statistical comparison of the iAUC between the large servings of the apple test foods and the fructose control are shown in Table 7.
Table 7. Adjusted* difference in iAUC between large servings of apples and apple juice, compared with the fructose control

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean change iAUC (µmol/L•h)</th>
<th>95% CI</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple (410 g versus fructose control)</td>
<td>-468.1</td>
<td>-1346.5, 410.4</td>
<td>0.286</td>
</tr>
<tr>
<td>Apple Juice (340 mL versus fructose control)</td>
<td>-592.0</td>
<td>-1373.4, 189.7</td>
<td>0.132</td>
</tr>
</tbody>
</table>

*Adjusted for order, timing, alcohol, smoking, family history of gout, and sex.

P ≤ 0.05 was considered significant.

All servings contained 26.7 g of fructose.

There was no significant difference between the intervention foods and control groups plasma uric acid response to fructose.

1.25 Satiety

Results from the VAS, assessing hunger, satisfaction, fullness, and prospective food consumption are presented in Table 8a and Table 8b. Each question and scale with both negative and positive extremes is presented in the table with the mean change in satiation after consumption of the test food. By using the scale beneath each question, the direction the mean is progressing towards can be seen. Each scale was numbered 0 to 100 mm left to right.

Mean change in response from each group was calculated for each VAS. Two time periods were isolated between baseline and 30 minutes (first 30 minutes), and 30 and 60 minutes (second 30 minutes). The mean change in response to both apple and apple juice servings within the aforementioned time periods was included to compare the satiety ratings.
Table 8a. mean differences between small servings of whole apples and apple juice groups of questions measuring satiety

<table>
<thead>
<tr>
<th>Questions</th>
<th>Apple (mean change*)</th>
<th>Apple Juice (mean change*)</th>
<th>Mean difference between Apple and Apple juice</th>
<th>95 % CI</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How hungry do you feel?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have never been more hungry – I am not hungry at all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>15.1</td>
<td>-4.1</td>
<td>-19.2</td>
<td>-39.8, 1.3</td>
<td>0.065</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-14.9</td>
<td>-10.7</td>
<td>4.2</td>
<td>-9.8, 18.3</td>
<td>0.545</td>
</tr>
<tr>
<td><strong>How satisfied do you feel?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I cannot eat another bite – I am completely empty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>-25.3</td>
<td>-2.9</td>
<td>22.4</td>
<td>7.9, 36.9</td>
<td>0.003</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>13.3</td>
<td>7.0</td>
<td>-6.4</td>
<td>-16.7, 4.1</td>
<td>0.223</td>
</tr>
<tr>
<td><strong>How full do you feel?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not at all full – Totally full</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>31.5</td>
<td>1.7</td>
<td>-29.8</td>
<td>-46.0, -13.6</td>
<td>0.001</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-13.0</td>
<td>-2.1</td>
<td>10.9</td>
<td>-2.8, 24.5</td>
<td>0.116</td>
</tr>
<tr>
<td><strong>How much do you think you can eat?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing at all – A lot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>-13.8</td>
<td>6.7</td>
<td>20.5</td>
<td>5.5, 35.6</td>
<td>0.009</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>9.9</td>
<td>1.4</td>
<td>-8.5</td>
<td>-17.6, 0.6</td>
<td>0.066</td>
</tr>
<tr>
<td><strong>How comfortable do you feel?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very uncomfortable – Very comfortable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>7.9</td>
<td>-1.2</td>
<td>-9.1</td>
<td>-23.6, 5.4</td>
<td>0.212</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-0.1</td>
<td>-1.1</td>
<td>-1.0</td>
<td>-14.3, 12.3</td>
<td>0.881</td>
</tr>
</tbody>
</table>
Table 8b. Mean differences between large servings of whole apples and apple juice groups of questions measuring satiety

<table>
<thead>
<tr>
<th>Questions</th>
<th>Apple (mean change*)</th>
<th>Apple Juice (mean change*)</th>
<th>Mean difference between Apple and Apple Juice</th>
<th>95 % CI</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>How hungry do you feel?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>31.1</td>
<td>8.4</td>
<td>-22.7</td>
<td>-39.2, -6.2</td>
<td>0.008</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-13.6</td>
<td>-14.1</td>
<td>-0.5</td>
<td>-15.2, 14.2</td>
<td>0.948</td>
</tr>
<tr>
<td>How satisfied do you feel?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>-51.6</td>
<td>-8.4</td>
<td>33.2</td>
<td>16.0, 50.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>15.2</td>
<td>11.9</td>
<td>-3.3</td>
<td>-14.6, 8.1</td>
<td>0.560</td>
</tr>
<tr>
<td>How full do you feel?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>49.8</td>
<td>20.6</td>
<td>-29.1</td>
<td>-47.1, -11.2</td>
<td>0.002</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-16.0</td>
<td>-14.1</td>
<td>1.9</td>
<td>-12.7, 16.6</td>
<td>0.789</td>
</tr>
<tr>
<td>How much do you think you can eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>-34.3</td>
<td>-4.0</td>
<td>30.3</td>
<td>15.1, 45.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>10.3</td>
<td>10.1</td>
<td>-0.2</td>
<td>-11.9, 11.5</td>
<td>0.970</td>
</tr>
<tr>
<td>How comfortable do you feel?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st 30 minutes</td>
<td>54.7</td>
<td>-5.1</td>
<td>-0.1</td>
<td>-17.3, 17.2</td>
<td>0.995</td>
</tr>
<tr>
<td>2nd 30 minutes</td>
<td>-5.1</td>
<td>2.2</td>
<td>-1.6</td>
<td>-13.6, 10.5</td>
<td>0.796</td>
</tr>
</tbody>
</table>

* P < 0.05 was considered significant.
First 30 minutes refers to the difference in satiety between baseline (0 minutes) and 30 minutes, the test food was consumed after the baseline satiety questionnaire was filled out. Second 30 minutes refers to the time between 30 and 60 minutes.
Small serving refers to either 205 g of apple or 170 mL of apple juice.
Large serving refers to either 405 g of apple or 340 mL of apple juice.
*Mean change from average rating at baseline to average rating at 30 minutes, or from average rating at 30 minutes to average rating at 60 minutes.
Apple (n = 23), Apple Juice (n = 21)
The appetite ratings were from 0 to 100 mm (left to right)
There were movements in the mean satiety ratings after consumption of the apple and apple juice. For example, after the small serving of apple there was a tendency for hunger ratings to increase by 15.1 mm on the 100 mm scale towards “I am not hungry at all” (Table 8a). In contrast, the mean change in the apple juice group was -4.1 mm in the direction of “I have never been more hungry”. The difference between the two foods was compared giving a 19.2 mm separation in hunger, although in the case of the small servings of apple and apple juice, this did not result in a significant difference in hunger ratings ($P = 0.065$).

During the second 30 minutes, there was a tendency for hunger ratings to shift 14.9 mm towards “I have never been more hungry,” after consumption of a small apple serving. The mean change indicates feelings of hunger were returning within 60 minutes (Table 8a).

After the large serving of apple, hunger ratings decreased by -31.1 mm on the 100 mm scale towards “I am not hungry at all” (Table 8b) during the first 30 minutes. The mean change in the large apple juice group was 8.4 mm, also in the direction of “I am not hungry at all”. The difference between the two foods was compared giving a -22.7 mm separation in hunger, which was significantly different ($P < 0.001$). This data indicated the apple group felt less hungry than the apple juice group during the first 30 minutes.

The mean change in satisfaction, fullness, and prospective food consumption VAS scales indicated appetite decreased after consumption of the small and large servings of apple compared to the servings of apple juice, during the first 30 minutes. While appetite ratings increased after both servings of apple and apple juice at 60 minutes, the apple juice group tended to have greater appetite ratings. None of the participants felt uncomfortable at any time point after consumption of the foods.
Discussion

The primary aim of this study was to assess whether the uric acid raising potential of fructose differed between a fructose monosaccharide beverage and the consumption of fructose present in apples and apple juice. A secondary aim was to test if whole apples induced greater satiation than juice. It has been suggested the natural ‘intrinsic’ fructose from fruit is as deleterious as added fructose (2). Others have suggested natural fructose is ‘healthier’, differing in its effects on blood uric acid compared to foods with ‘added’ fructose (1). Because of this, we expected the intervention foods (apple and apple juice) to have an attenuated impact on acute uric acid concentration compared to the control fructose monosaccharide beverage. Conversely, the intervention foods and control fructose beverage did not differ significantly in their effect on uric acid concentration, although all responses were small and transient.

Our primary findings suggest the uric acid response was independent of the food providing the fructose dose. Our secondary finding was that the consumption of whole apples appeared to cause a greater suppression of appetite compared with apple juice. Compared to the apple juice group, the apple group reported reduced hunger, greater satisfaction, fullness and a reduced desire for prospective food consumption. The tested volumes were realistic of normal consumption, and provide a useful evaluation of the effects of fructose containing fruit and fruit products on acute uric acid production and satiety. A review of current literature suggests the present study is the first in New Zealand to compare the acute uric acid production from whole apples and 100 % apple juice in realistic serving sizes as far as we are aware.

Studies were previously undertaken on this topic using large doses of fructose, unrealistic of regular consumption (7, 15, 16). Perez-Pozo et al. (6) fed participants 200 g fructose per day and observed the onset of the metabolic syndrome within a two-week period (6). The mean uric acid concentration rose by $65 \pm 5 \mu\text{mol/L}$ (6), a much larger increase than observed in the
present study. Although this evidence is helpful to determine the physiological effect of extreme levels of fructose intake, it does not provide insight into what effects commonly consumed fructose amounts might induce in the general population (18).

Older studies observed a moderate increase in serum uric acid after supplementation of 1 g per kg body weight of fructose (81). Our findings agree with the conclusions determined during 1970, that fructose was responsible for the increase in plasma/serum uric acid concentration (112, 113). A 50 g (0.67 – 0.83 g/kg) intravenous fructose dose decreased the ATP content of the liver to 50 % within 30 minutes post consumption, inducing a 64 μM (64 μmol/L) uric acid rise (53). In contrast, a 0.5g/kg intravenous dose of fructose did not induce any significant changes in blood uric acid, suggesting a dose-response is present during fructose consumption (114). Consequently, 0.5 g/kg was determined a safe dose to administer patients in a clinical setting (114). In line with the mean weight of participants, we administered a mean oral dose of 0.4g/kg body weight of fructose within the present study, and observed a significant rise in uric acid concentration.

Many human studies have investigated the antioxidant properties of apples and fruit juices (7, 8, 15, 16, 75, 115-117); and noted an increase in the antioxidant status of participants with as little as 150 ml of apple juice (118). The rise in antioxidant status seen in studies that have not measured uric acid concentrations is suggested to be augmented by the fructose to uric acid reaction (8). Lotito et al. observed a 31 μM (31 μmol/L) increase in urate concentration after 0.83g/kg fructose from five apples (63.9 ± 2.9 g) (15). Their study concluded a transient rise in plasma urate was common after consumption of fructose-containing food (16, 119). Contrary to our findings, Briviba et al. noted a 40 μmol/L increase in uric acid concentration after administering participants 1000 g of Golden Delicious apples (7).

However, smaller fructose doses (36 g) have also induced a small, transient but not significant rise in serum uric acid at 60 minutes (16). The present study did not measure beyond 60
minutes post-consumption due to time restrictions, but the mean change in uric acid appeared to remain elevated after consumption of apple juice at the conclusion of the study period. Data suggests we might have observed a longer uric acid response if we had continued measurement until 90 minutes (8).

Vieira et al. (75) administered a similar volume of apple juice to that in the present study using five pressed apples; without specified fructose dose (75). Apple juice consumption was associated with an increase in serum uric acid concentration, without a dose response to fructose (75). In contrast to this recent finding, we did not observe a similar uric acid response after differing amounts of fructose. Instead, the change in uric acid concentration increased with increasing fructose administration. These results suggest a dose response effect with increasing consumption of fructose as seen elsewhere (120). We question the significance of these results to application in a clinical setting, as the transient increases observed were not large enough to warrant concern of hyperuricemia risk in our cohort of young healthy adults at low risk of gout. Rises in plasma uric acid may have been more significant, or clinically significant in those at risk or with gout.

The benefits of whole fruits (antioxidants, potassium, fibre and other compounds) might outweigh the negative effects of fructose from beverages (1). Fruit juice is not recommended as a substitute for whole fruit intake (21, 36), despite it being viewed by many as a healthy alternative to sugar-sweetened beverages. A large cohort study recently found no association with fruit and the risk of gout (3). The Ministry of Health predicts sugary non-alcoholic beverages (including fruit juice) provide 26% and 29% of the total sugar consumed by New Zealand children and adults respectively (43, 66), whereas fruit only comprised 13 % consumed by adults (17)
1.26 Satiation

A secondary finding was whole apples stimulated greater satiety and slower appetite return than apple juice. Studies that observed this result also determined the rate of ingestion increased when fruit juice was consumed (22, 23, 25, 103, 121, 122) and less satiation occurred compared to whole fruit (23, 25). There is evidence that energy from liquid sources is less satiating and is not well compensated for by eating less at successive meals (24, 123). Mattes suggests this is due to the lack of gastric distension, decreased transit time, and the body’s lack of detection of calories from liquids (93). Although not all studies have observed a difference between solid and liquid versions of foods (91, 124).

Matching the energy and fibre contents between the intervention foods was not possible in the present study. An increase in fibre and energy present in test foods, particularly the whole apple is suggested to increase satiety (22, 23). Yet it was noted when the fibre contents of both liquid and solid test foods were matched; there were no significant differences between satiety rating (25). Pectin fibre from a sweetened beverage repressed appetite and ghrelin secretion, but did not decrease successive food consumption (122), compared to pectin fibres consumed from solid fruit (122). We matched fructose content between test foods; therefore, these findings are novel as the effects on satiety of foods matched for natural fructose content has not been considered in previous literature.

We used cloudy apple juice with the fibre content retained, although mechanically disrupted (106). The average dietary fibre content of whole raw apple is 2.1 g per 100 g (73), compared to 0.3 g per 100 g in the commercial apple juice (106). We did not compare the satiating properties of the intervention foods to the fructose monosaccharide; thus, the effect of fibre cannot be ruled out in changes observed in satiation, due to the difference in fibre content. The fructose monosaccharide is also suggested to decrease satiety (41), as it was noted that fructose does not induce the same hormonal regulatory effects as glucose (41). Because we
matched the fructose concentrations between the foods, it could be expected that the volume of food or fibre had the greatest effects.

The solid structure of food may be the main contributing factor to increased satiety, as solid meals are noted to induce different physiological responses when compared to a homogenised form (103). It is suggested solid foods increase cephalic phase, stomach distension and mechanical stimulus, as they are of a larger food particle size (103). However, when weight, fibre and energy content were matched, the volume of the whole apple was noted as larger (25). The due to the solid structure of fruit, the cell wall is likely to increase chewing quantity (23), which was noted to increase the length of the cephalic phase during digestion (125-127). An increased cephalic phase may induce a longer satiety response (125). Accordingly, it has been determined as difficult to separate the effects of the solid structure of the food from the effect of fibre from the same food on satiety in previous studies (22).

1.27 Strength and limitations

The serving sizes used in this study strengthen its relevance to dietary practice. The amounts we provided were below (small serving) and above (large serving) the recommended servings of fruit per day in New Zealand (42), equating to approximately 1.6 and 3.2 apples. The recommended serving size on the nutrition information panel of the apple juice used was 200 mL, however; we provided participants 170 mL and 340 mL servings. Two hundred millilitres is less than a standard cup size; thus it is likely consumers would consume more than the recommended serving size. Therefore, the servings administered in the present trial were likely to be realistic. In the current study, no participants struggled to consume the serving amounts of juice.

We used a positive and a negative control. Our positive control was used to note the effects of the fructose monosaccharide without other effect from other food components. In the monosaccharide form, it does not require further digestion before absorption takes place.
(107); thus, quick absorption was predicted. Glucose was used as a negative control, as there is no evidence pointing towards glucose being involved in uric acid metabolism (53, 108). Consequently, we did not expect to see an increase in uric acid after consumption of the glucose. Other strengths include participant compliance with pre-test requirements, consistency during blood measurements, accurate timing methods, and few missing data.

There are limitations to this study. The inability to blind participants to which intervention they were receiving was a limitation. A further limitation originates from the use of a cohort selected from predominantly female New Zealand European university students, enrolled in nutrition papers. In contrast, gout is more likely to occur in older men (14, 86-88); thus it is unknown how applicable these findings are to the metabolically at risk population. Previous studies have suggested the risk of gout is also linked to the presence of several genes common in Māori and Pacific (30). As the study population had only a small proportion of Māori (6 %) and Pacific (2 %) we did not have the power to measure any differences in uric acid response between ethnicity.

Although VAS are a valid measurement tool (128), satiety is a subjective perception (22) perhaps limiting reliability. We measured satiety through the VAS method and did not adjust for any covariates or test ad libitum consumption, which is often used in satiety studies (128). Confounders to these satiety measurements are likely to be the differing energy content, mass and fibre content of the apples and apple juice, as apples are fibrous, “energy dilute” foods in comparison to apple juice (36).

Lastly, discrepancies exist between the mean difference of uric acid (Table 5) and the iAUC data (Table 6 and 7). Data outliers in the control group were not removed without valid justification (Table 7), and are the likely cause. The results from these participants might be outliers due to flawed methodology or might be legitimate results. These people might be representative of a small component of the population who are be susceptible to the effects of
fructose on uric acid, which our sample size was too small to detect. As we had a small sample and outliers present, the distribution is likely moved away from the true mean, skewing the data.

1.28 Conclusions and recommendations

Gout is an important health issue for New Zealanders, particularly in those of Pacific or Māori descent and is increasing in prevalence (13, 31). We noted a significant, but not clinically significant increase in uric acid in a cohort of young university students. The rise in uric acid was transient and not likely to induce negative consequences in our cohort. What effect natural fructose would have on uric acid concentration in an older population with greater genetic or ethnic susceptibility (86), we cannot provide comment. We determined no difference in uric acid response to fructose from natural sources in realistic serving sizes when compared with a monosaccharide beverage. Whole apples were determined to be more satiating than apple juice in this cohort of healthy young New Zealanders. This study provides further evidence for consumption of whole fruit rather than fruit juice.

We recommend further investigation into the difference between natural versus added fructose in an older population. As our cohort were predominantly female university students, the results of this study cannot be generalised to other subpopulations, which might be at greater risk of gout (14, 86). A similar study could be conducted with a greater cohort of Māori and Pacific peoples and people of more advanced age, with consideration to the known genetic factors in hyperuricemic development.
Implications to dietetic practice

Although we observed a significant increase in plasma uric acid concentration in this randomised controlled crossover trial, the implications of these results may bear little clinical significance. The participants were typically healthy students in their early twenties, who were not at risk of gout. Whether the current study has relevance to populations who more metabolically at risk of gout is undetermined. More evidence is required to broadly apply these results to the larger or more ethnically diverse demographic, which might be seen in dietetic practice.

The popular idea that natural fructose behaves in a different manner in the body was determined to be a misconception in the present study. No statistical difference was observed between the effects of a fructose control beverage, whole apple or apple juice on plasma uric acid concentration. The increase in uric acid was dependent on the dose of fructose, not the food or beverage form.

We observed a small transient rise in plasma uric acid concentration. For a young, healthy weight population there was no suggestion this dose–response is of pathological concern. Although fruit contains fructose, it is still a low energy; high nutrient dense appropriate food (42), which has beneficial effects as a source of fibre, vitamins and satiation within the diet. Many foods have been determined to induce gout attacks including meat, seafood and some alcohol (84, 129). However, fruit has not been observed to induce gout attacks as far as we are aware. Accordingly, the risks and benefits need to be weighed up when considering what foods to include and exclude for the dietary management of gout. The restriction of foods should be considered on an individual basis.

The dietetic message surrounding the intake of sugary beverages including fruit drinks should not change. It is best to choose 100% fruit juice without added sugar. However, fruit juices
contain similar nutrients present in the whole fruit, but may contribute more energy, less fibre
and other favourable components i.e. antioxidant properties as whole fruit (21, 130). We have
observed further evidence supporting existing recommendation that whole fruit induces
greater satiation than apple juice, providing greater evidence for whole fruit consumption to
be encouraged over fruit juices (36). Benefits such as increased fibre and effects on satiety
antioxidants and phenolic compounds and vitamins need to be considered in the context of
whole fruit in the diet, not solely as a source of fructose. Carbohydrates in a liquid matrix are
not as well compensated as solid forms, at subsequent meals; which may promote positive
energy balance and weight gain (24). The consumption of fruit juice might lead to a greater
energy intake, as one glass has greater calories without the benefits of dietary fibre compared
to fruit (21, 130). It is suggested there is no dietary requirement for fruit juice consumption
(21, 131), as whole fruit provide a better balance of energy and satiation (21, 131).

An aim of incorporating more satiating foods including fruit and vegetables into the diet is to
induce weight loss. The consumption of satiating foods is correlated with reduced energy
intake (132, 133). Strategies to avoid obesity set by the World Health Organization include
promoting fruit and vegetable intake and restricting intake of energy dense foods (36).
Increasing the intake of "energy dilute" foods including fruits, could decrease total energy
intake and increase micronutrient intake (1, 36), but also reduce the risk of disease associated
with obesity and its related co-morbidities (1).

Dietitians need to be aware of the misconception some patients might hold; that free fructose
that from natural sources is treated differently in the body, and to promote fruit intake.
Educating patients about the benefits of fruit consumption, including key nutrients and a
reduced risk of disease is the first step.
Literature Cited


80. Edwards NL. The role of hyperuricemia and gout in kidney and cardiovascular


Malagelada JR, Go VL, Summerskill WH. Different gastric, pancreatic, and biliary responses to solid-liquid or homogenized meals. Digestive diseases and sciences. 1979;24(2):101-10.


Appendices

A. Ethics Application related documents:
   - Ethics application form
   - Participant information sheet
   - Participant consent form
   - Māori Consultation
   - Demographic questionnaire

B. Ethical approval
   - Amendment letter
   - Ethical approval letter
   - Māori consultation letter of approval

C. Food composition analysis
   - Cawthron Laboratory certificate of analysis
   - Reference for laboratory protocol

D. Communication strategy

E. Blood collection protocol

F. Second test session adherence questionnaire

G. Example satiety questionnaire booklet

H. Uric acid analysis
   - Dilution curve
   - UA2 test kit reagent
Appendix A

Ethics application form
Participant information sheet
Participant consent form
Māori Consultation
Demographic questionnaire
UNIVERSITY OF OTAGO HUMAN ETHICS COMMITTEE
APPLICATION FORM: CATEGORY A

Form updated: May 2014

1. University of Otago staff member responsible for project:
   
   Surname    First Name    Title
   Venn        Bernard       (Dr)

2. Department/School:
   Human Nutrition

3. Contact details of staff member responsible (always include your email address):
   bernard.venn@otago.ac.nz
   Tel 03 479 5068

4. Title of project: HUNT311 clinical nutritional laboratory; a repeated teaching activity

5. Indicate project type and names of other investigators and students:

   Staff Co-investigators    Names:  
   ____________________________                    ____________________________
   Tony Merriman
   Sara White MDiet
   Emma Carran MDiet
   Andrew Reynolds PhD

   Student Researchers    Names:  
   ____________________________                    ____________________________
   Level of Study (PhD, Masters, Hons):

   External Researcher    Names:  
   ____________________________                    ____________________________
   Institute/Company:

6. Is this a repeated class teaching activity?
   (Delete answer that does not apply)
YES

If YES and this application is to continue a previously approved repeated class teaching activity, provide Reference Number: 13/022

7. **Fast-Track procedure** *(Delete answer that does not apply)*

   Do you request fast-track consideration? *(See 'Filling Out Your Human Ethics Application')*

   NO

   If YES, provide a robust justification on the need for urgency:

8. **When will recruitment and data collection commence?**
   February 2015

   **When will data collection be completed?**
   April 2015

9. **Funding of project**
   Is the project to be funded by an external grant?
   NO

   If YES, specify who is funding the project:

   If commercial use will be made of the data, will potential participants be made aware of this before they agree to participate? If not, explain: No commercial use

10. **Brief description in lay terms of the purpose of the project** *(approx. 75 words):*

    The purpose of the HUNT311 laboratories is for students to experience participation in a clinical nutritional trial. Measured outcomes will be changes in blood glucose, feelings of hunger, and serum uric acid concentrations in response to consuming various carbohydrate containing foods. The laboratory will be a source of individual and group data to be used in a class assignment with potential for publication using anonymous group data. Genetic data will be collected to investigate the interaction between genotype and diet in determining serum uric acid and glucose concentrations – this information will be analysed by Human Nutrition MDiet students.

11. **Aim and description of project**

    The aim of this laboratory is to test the glycaemic, satiating and uric acid raising potential of
carbohydrate containing foods. This information will be used by HUNT311 students as a learning exercise and in the writing of his or her assignment. A secondary (non-teaching) aim is to investigate the interaction between genetic variants and dietary exposure in determining serum uric acid and glucose response. One existing example of this is non-additive interaction between the GLUT9 gene and sugar-sweetened beverage consumption in determining serum urate levels (Batt et al. 2013). This work will replicate published interactions with the potential to discover novel interactions.


12. Researcher/instructor experience and qualifications in this research area

Dr. Venn is experienced in conducting research trials involving human participants. Testing will be carried out according to our standard procedure in the Department of Human Nutrition Undergraduate Laboratories. A/Prof Merriman has extensive experience in researching genetics of metabolic disease, including environmental interactions.

13. Participants

13(a) Population from which participants are drawn: Human Nutrition students

13(b) Inclusion and exclusion criteria:

Inclusion: men and women in the age range of 18 – 60 y, inclusive.

Exclusion: People diagnosed with chronic disease including diabetes mellitus, cardiovascular disease, cancer, and diseases of the digestive system; that suffer from food allergies; and women who are pregnant.

13(c) Estimated number of participants: All HUNT311 students (currently 100+)

13(d) Age range of participants: 18-60y

13(e) Method of recruitment: Recruitment will be by invitation to the students by email and in class at the University of Otago.

13(f) Specify and justify any payment or reward to be offered

No payment or reward

14. Methods and Procedures:

The purpose and scope of the laboratory will be discussed in class. An Information Sheet (attached) will be given to students and teaching and research staff will be available to answer questions regarding the study. If students are willing to continue, a consent form (attached) will be given to
them. Participants will have their height and weight measured in a screened-off area to ensure the participants privacy. A questionnaire will be administered to ensure that eligibility criteria are met and for collection of demographic data. Test foods will be provided to participants. In 2015, the foods will be supermarket-purchased sugary beverages and whole fruit.

For measuring blood glucose and serum uric acid, capillary blood is collected by finger pricking using a sterilised disposable lancet. During each test, a series of eight blood samples are collected over a period of three hours following the consumption of the food. Each student will test two foods, each on a separate non-consecutive day. The Department of Human Nutrition will use trained personnel to do the finger pricking. Students will attend the laboratory after an overnight fast of at least 10 hours. On the evenings preceding each of these test days, participants will be advised not to exercise and to ensure that their evening meal contains a carbohydrate-rich food. On each of the test days, two finger-prick blood samples will be taken five minutes apart as a baseline blood glucose concentration. This method of collecting blood for analysis causes minimal discomfort to the participant. Blood glucose concentrations will be determined from a drop of blood using a Hemocue Glucose 201 Analyzer. Following this, a test food will be consumed over a fifteen minute period and a series of six more finger-pricks will be undertaken at 15, 30, 45, 60, 90 and 120 min. In the event of an abnormal result, a repeat fingerprick may be required. Adhesive plasters will be provided to hold in place a cotton wool swab covering the small incision. The total volume of blood extracted from the finger-pricks will be less than two millilitres. There is no excess blood for disposal. During this laboratory, students will also be given a set of questions regarding how hungry they feel as a measure of satiety. Blood pressure will be taken at baseline and one hour after consumption of the test food in accordance with the Department of Human Nutrition good clinical practice guidelines using an OMRON digital blood pressure monitor. If the average of three systolic blood pressure readings is less than 100 or greater than 140 mmHg, the student will proceed with the laboratory and will be advised to consult his or her healthcare provider.

For the genetic analysis, DNA will be extracted from a 10 ml saliva sample using a commercially available kit. The DNA will be quantitated, diluted to a standard concentration and genotyped for selected genetic variants using the Taqman genotyping system. Interaction between genotype and dietary exposure will be determined using linear regression in a statistical software package such as STATA, with inclusion of an interaction term.
15. Compliance with The Privacy Act 1993 and the Health Information Privacy Code 1994 imposes strict requirements concerning the collection, use and disclosure of personal information.

The questions below allow the Committee to assess compliance.

15(a) Are you collecting and storing personal information (e.g. name, contact details, designation, position etc) directly from the individual concerned that could identify the individual? (Delete the answer that does not apply.)

YES

15(b) Are you collecting information about individuals from another source?

NO

If YES, explain:

15(c) Collecting Personal Information (Delete the answer that does not apply):

• Will you be collecting personal information (e.g. name, contact details, position, company, anything that could identify the individual)?

YES

• Will you inform participants of the purpose for which you are collecting the information and the uses you propose to make of it?

YES

• Will you inform participants of who will receive the information?

YES

• Will you inform participants of the consequences, if any, of not supplying the information?

YES

• Will you inform participants of their rights of access to and correction of personal information?

YES

Where the answer is YES, make sure the information is included in the Information Sheet for Participants.

If you are NOT informing them of the points above, please explain why:

15(d) Outline your data storage, security procedures and length of time data will be kept

The information will remain confidential to the study investigators. Paper copies will be kept in a lockable office and electronic data stored on departmental computers in password protected files. The results of this study may be published but no individual's identity will be revealed. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.
15(e) Who will have access to personal information, under what conditions, and subject to what safeguards? If you are obtaining information from another source, include details of how this will be accessed and include written permission if appropriate. Will participants have access to the information they have provided?

Only Dr Bernard Venn will have permanent access to the personal information. Paper copies will be stored in Dr Venn's University of Otago office and any information transferred into digital form will be stored on Dr Venn's University computer. If a nominated postgraduate student enters data, this will only be done on a desktop university password-protected computer. At the completion of data entry, the student will be asked to transfer the electronic file to Dr Bernard Venn and to delete the file from the student computer.

Statistical analysis will be done using anonymous data.

15(f) Do you intend to publish any personal information they have provided?

NO

If YES, specify in what form you intend to do this:

15(g) Do you propose to collect demographic information to describe your sample? For example: gender, age, ethnicity, education level, etc.

Yes

15(h) Have you, or will you, undertake Māori consultation? Choose one of the options below, and delete the option that does not apply:

(Refer to http://www.otago.ac.nz/research/maoriconsultation/index.html).

NO If not, provide a brief outline of your reasons (e.g. the research is being undertaken overseas):

YES We have completed the University of Otago online Māori consultation form attached (pages 14-15). It is scheduled for discussion at the Ngāi Tahu Research Consultation Committee meeting 18th Nov.

16. Does the research or teaching project involve any form of deception?

NO

If yes, explain all debriefing procedures:

17. Disclose and discuss any potential problems or ethical considerations: (For example: medical or legal problems, issues with disclosure, conflict of interest, safety of the researcher, etc. Note: if the student researcher will be travelling overseas to undertake the research, refer to item 12
of the *Filling Out Your Human Ethics Application* document. Please note that approval from the Human Ethics Committee does not override the University of Otago’s Field Policy and Travel Policy, which must be complied with.)

This is a repeated teaching activity and research students may be involved in data collection and analysis from year to year. The research students will only work with data with the University of Otago student ID as an identifier, rather than student names.

There may be some discomfort from finger pricking

18.  *Applicant's Signature:* .................................................................
     *Name (please print):* .................................................................
     *Date:* ..............................
     *The signatory should be the staff member detailed at Question 1.*

19.  **Departmental approval:**  *I have read this application and believe it to be valid research and ethically sound. I approve the research design. The Research proposed in this application is compatible with the University of Otago policies and I give my consent for the application to be forwarded to the University of Otago Human Ethics Committee with my recommendation that it be approved.*
     **Signature of **Head of Department:** ............................................
     **Name of HOD (please print):** ...................................................
     **Date:** .................................................................
     **Where the Head of Department is also the Applicant, then an appropriate senior staff member must sign on behalf of the Department or School.**
HUNT311 clinical nutritional laboratory; a repeated teaching activity

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?

The aim of this study is to test the glycaemic, satiating properties and uric acid raising potential of carbohydrate containing foods. This requires attending the laboratory on two occasions. You and other HUNT311 students will use the information in the writing of a HUNT311 assignment. If you choose not to participate, you will still be required to attend the laboratory to observe and data will be provided to you; the assessment of your assignment will in no way be affected.

What Type of Participants are being sought?

HUNT311 students with no diagnosis of diabetes mellitus, cardiovascular disease, cancer, diseases of the digestive system; you are not pregnant, you do not suffer from food allergies or take medication that affects glucose absorption and metabolism.

What will Participants be Asked to Do?

Main laboratory experiment

You will be asked to attend the Department of Human Nutrition Undergraduate Laboratory on two occasions, separated by one or two weeks apart, as you will be testing two different amounts of fruits or beverages. If eligibility criteria are met, you will be asked to read and sign a consent form, we will collect some personal information from you comprising demographics, height and weight. Following this, the first test will be conducted. Testing is conducted in the morning with a start time of between 7 - 8 am. You will be required to fast, ie: to have no food, no sugar-sweetened chewing gum or drinks except water after 10 pm on the night before the test. We would prefer that you did not walk to the University. If you do walk or cycle we would like you to arrive 20 minutes early so that your heart rate and blood glucose have a chance to settle down before you start the test. On arrival your blood pressure will be taken following which two finger-prick blood sample will be taken in the fasting state using a single-use disposable lancet designed to minimize discomfort. You will then be given a test food or beverage to consume. After this, additional finger-prick blood samples will be taken at 15, 30, 45, 60, 90, and 120 min. The fingerpricks may cause some discomfort. In the event of an abnormal result, a repeat finger-prick may be required. The total volume of blood collected will amount to less than half a teaspoon. During this three hours we would like you to remain seated in the room with the exception of toilet visits if necessary. You are free to read or talk. At the end of three hours there will be some food for you to eat on the premises or to take away.

Genotyping uric acid and glucose genes

You will be asked to donate a saliva sample from which DNA will be prepared. This will be used to investigate how genes involved in regulating serum uric acid and glucose levels interact with diet in their regulation of uric acid and glucose levels. This research will not be part of your laboratory, but will contribute to the research projects of Masters of Dietetics students within the
Department of Human Nutrition. Please note: you will not be required to write up this aspect of the laboratory. If you agree to take part we will advise you of your genotype for the glucose and uric acid transporter GLUT9.

**What Data or Information will be Collected and What Use will be Made of it?**

For the main laboratory exercise we will collect data on your age, ethnicity, smoking habits and gender and we will be measuring your height and weight. The purpose of collecting this information is to describe the overall characteristics of the study population. We will also ask you to fill in a medical questionnaire to ensure you meet the study eligibility criteria. From your blood samples we will be testing glucose and uric acid concentration. The information will remain confidential to the study investigators. Paper copies will be kept in a locked office and electronic data stored on a departmental computer. The results of the project will be pooled and may be published and available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve your anonymity. The data and samples collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. Data and samples obtained as a result of the research will be retained for at least 5 years in secure storage. Any personal information held on the participants such as contact details may be destroyed at the completion of the research even though the data and samples derived from the research will, in most cases, be kept for much longer or possibly indefinitely. If you choose not to supply information this may exclude you from taking part in the study. You have rights of access to the personal information that you have given to us and you may correct or change this information.

For the genetic testing, we will genotype your GLUT9 gene to investigate the interaction between this genetic variant and dietary exposure in determining serum uric acid and glucose response.

Interaction between gene variants and dietary exposure is an emerging field and we would like to retain your DNA if new gene interactions are found relating to serum uric acid and glucose response.

Testing blood glucose has the potential to reveal whether a person has diabetes or is at risk of pre-diabetes. If elevated blood glucose concentrations are found, you will be advised to make an appointment with student health or with your general practitioner. If the average of three systolic blood pressure readings is less than 100 or greater than 140 mmHg, you will be able to proceed with the laboratory but we will advise you to consult your healthcare provider.

**Can Participants Change their Mind and Withdraw from the Project?**

You may withdraw from participation in the project at any time and without any disadvantage to yourself or to your HUNT311 assessment of any kind.

**What if Participants have any Questions?**

If you have any questions about our project, either now or in the future, please contact -

Dr Bernard Venn; Department of Human Nutrition

Telephone: 03 479 5068 email bernard.venn@otago.ac.nz

Associate Professor Tony Merriman; Department of Biochemistry
Telephone: 03 479 5798 email tony.merriman@otago.ac.nz

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet and understand the procedures. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

1. My participation in the project is entirely voluntary;
2. I am free to withdraw from the project at any time without any disadvantage to myself or to my HUNT311 assessment;
3. Personal identifying information will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for at least five years;
4. Fingerprick blood sampling may cause some discomfort.
5. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand), but every attempt will be made to preserve my anonymity.

I consent to attending the laboratory on two days following an overnight fast, consuming the study food and providing eight blood samples obtained by finger pricking over three hours on each test day

Yes ☐ / No ☐

I consent to providing a saliva sample for genotyping

Yes ☐ / No ☐

I consent to storage of my saliva sample for future genotyping relating to uric acid and glucose metabolism

Yes ☐ / No ☐

Name ........................................... Signature ............................................... Date ......................

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Principal Investigator 1
Name: Dr Bernard Venn
Department: Department of Human Nutrition
Campus: DUNEDIN
Email: bernard.venn@otago.ac.nz Telephone: Not Supplied

Principal Investigator 2
Name: Aspro Tony Merriman
Department: Department of Biochemistry
Campus: DUNEDIN
Email: tony.merriman@otago.ac.nz Telephone: Not Supplied

Is this Otago District Health Board research?
No
Does this research involve human participants?
Yes
Description in lay terms of the proposed research
The purpose of the HUNT311 laboratories is for students to experience participation in a clinical nutritional trial. Measured outcomes will be changes in blood glucose, feelings of hunger, and serum uric acid concentrations in response to consuming various carbohydrate containing foods. The laboratory will be a source of individual and group data to be used in a class assignment with potential for publication using anonymous group data. Genetic data will be collected to investigate the interaction between genotype and diet in determining serum uric acid and glucose concentrations.

Description in lay terms of the potential outcomes of the area of research
The aim of this laboratory is to test the glycaemic, satiating and uric acid raising potential of carbohydrate containing foods. This information will be used by HUNT311 students as a learning exercise and in the writing of his or her assignment. A secondary (non-teaching) aim is to investigate the interaction between genetic variants and dietary exposure in determining serum uric acid and glucose response.

Potential areas that are of interest to or of concern for Māori
The prevalence of gout among Māori is one of the highest in the world (Te Karu et al. Māori experiences and perceptions of gout and its treatment: a kaupapa Māori qualitative study. J Prim Health Care 20135214-222). To help alleviate this painful problem it is important to explore whether there is a genetic basis. One such candidate is SLC2A9 a gene which encodes the GLUT9 glucose fructose and uric acid transport protein in the kidneys. The protein takes one of two forms with one form being more protective of gout than the other. Gout is caused by chronic elevation of uric acid in the blood. One source of uric acid is a high intake of the sugar fructose. Table sugar (sucrose) comprises half fructose and half glucose therefore fructose can be consumed in large amounts when people drink sugary beverages. In this class of nutrition students (approx 100) there will be some Māori students. Our intention is to ask the students to consume a source of fructose (a sugary beverage or whole fruit) to measure the change in serum uric acid and to test how this change relates to the GLUT9 phenotype. Identifying this interaction could help our understanding of a mechanism linking fructose consumption to risk of gout. DNA will be extracted from a saliva sample.

Collaborations in this area of research
This is a University of Otago project involving the Departments of Human Nutrition and Biochemistry without external collaborators
Potential funding bodies
University funded

Location
University of Otago human nutrition undergraduate laboratory

Other relevant information
Reference_17775
**Information Collection Sheet – Week 1**

The information below will help us better understand you and your test results. This information is voluntary, if you do not wish to answer any question you may abstain. The information you provide will be de-identified and pooled with the results of every other participant to describe the group characteristics. This information collection sheet will be used to retrieve relevant details for research purposes by the investigators, and then stored securely for up to 5 years.

Student ID Number: ___________________________ Male/ Female

Date of Birth: ________________ Contact phone number: ________________

Have you eaten anything from 10pm last night? ________________ YES/NO

If yes, what have you eaten since 10pm last night, and when?

Did you have a carbohydrate rich meal for dinner last night? ________ YES/NO

Did you drink alcohol yesterday or this morning? ________________ YES/NO

If yes, how many standards did you consume? _____

Did you do any physical activity outside of your normal routine yesterday or this morning? _____________________ YES/NO

If yes, what did you do?

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

☐ New Zealand European

☐ Māori

☐ Samoan

☐ Cook Island Māori

☐ Tongan

☐ Chinese

☐ Indian

☐ Other – Please specify: ________________

Smoking status:

☐ Never smoked

☐ Previous smoker

☐ Current smoker – How many cigarettes per day? ________

To your knowledge is there a history of gout in your family? ________ YES/NO

Are you pregnant? (if female) _____________________________ YES/NO

Have you been diagnosed with diabetes, heart disease, stroke, cancer? __ YES/NO

Or diagnosed with any disease of the digestive system? ____________ YES/NO
Please list any current medicines you are taking, frequency and dose:

Please list current supplements you are taking, brand and frequency:

Please list any food allergy or intolerance:

How many servings per week would you normally consume of soft drink/energy drinks? (1 serving = 1 glass = 250ml) (do not include diet or sugar-free drinks):

☐ 2+ servings a day
☐ 1 serving per day
☐ 5-6 Servings per week
☐ 3-4 servings per week
☐ 1-2 servings per week
☐ 1-2 servings per month
☐ Never

How many servings per week would you normally consume of fruit juice? (1 serving = 1 glass = 250ml)

☐ 2+ servings a day
☐ 1 serving per day
☐ 5-6 Servings per week
☐ 3-4 servings per week
☐ 1-2 servings per week
☐ 1-2 servings per month
☐ Never

**Blood Glucose Readings – Week 1**
You will have 3 blood glucose tests done, please record the readings below;

Baseline:

30 minutes:

60 minutes
Appendix B

Amendment letter

Ethical approval letter

Māori consultation letter of approval
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 14/204.

Thank you for your request for amendment to add blood pressure as a measurement into this year’s HUNT 311 laboratory. In addition, you have added Natasha Rodrigues, an MSc student, to the project due to her skills with carbohydrate and fructose metabolism.

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
Dr B Venn  
Department of Human Nutrition  
Division of Sciences

27 November 2014

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 14/204.

Thank you for your email responding to the Committee, for and providing the revised Information Sheet and Consent Form.

Thank you for clarifying the information for participants regarding the two aspects of the study, what they are being asked to consent to and what they will experience, and procedures in case of accidental findings. Thank you for providing three options on the Consent Form to allow participants to consent the main laboratory experiment, the saliva sample for genotyping, and the storage of the DNA for possible future testing.

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

As this is a repeated teaching activity, approval is for up to three years from the date of this letter. If the repeated teaching activity will continue beyond three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

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

cc. Professor S Samman  
Department of Human Nutrition
Tuesday, 18 November 2014.

Dr Bernard Venn,
Department of Human Nutrition,
DUNEDIN.

Tēnā Koe Dr Bernard Venn,

HUNT311 clinical nutritional laboratory; a repeated teaching activity

The Ngāi Tahu Research Consultation Committee (the committee) met on Tuesday, 18 November 2014 to discuss your research proposition.

By way of introduction, this response from The Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum it states "Ngāi Tahu acknowledges that the consultation process outline in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee base consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon; adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

The Committee notes this is a class laboratory exercise but also notes it is dealing with some important aspects for Māori health. The Committee suggests that Māori health issues are outlined as part of this class to discuss important health disparities.

We wish you every success in your research and the committee also requests a copy of the research findings.

This letter of suggestion, recommendation and advice is current for an 18 month period from Tuesday, 18 November 2014 to 18 May 2016.

The Ngāi Tahu Research Consultation Committee has membership from:

Te Rūnanga o Īhūkua Incorporated
Kāti Huirapa Rūnaka ki Paketeraki
Te Rūnanga o Moeraki
NGÄI TAHU RESEARCH CONSULTATION COMMITTEE

TE KOMITI RAKAHAU KI KÄI TAHU

Nähaku noa, nā

Mark Brunton
Kaiwhakahaere Rangahau Mäori
Research Manager Mäori
Research Division
Te Whare Wänanga o Otago
Ph: +64 3 479 8738
Email: mark.brunton@otago.ac.nz
Web: www.otago.ac.nz

The Ngäi Tahu Research Consultation Committee has membership from:

Te Ränanga o Ōtäkou Incorporated
Käitihuirapa Ränaka ki Pukeahuak
te Ränanga o Moeraki
Appendix C

Cawthron Laboratory certificates of analysis

Reference for laboratory protocol
Certificate of Analysis: Final

Project Number: T47707

University of Otago
PO Box 56
DUNEDIN
Attention: Sara White

Customer Order No: 9829FM
Email Recipients: Sara White

Sample Details
Laboratory ID: T47707-1
Sample Type: Product
Description: Whole Raw Apples x 5 (Royal Gala)
Date Sampled: 11/02/2015 10:30
Date Received: 12/02/2015 08:20

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Result</th>
<th>Units</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose*</td>
<td>5.2</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Glucose*</td>
<td>1.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>2.6</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
</tbody>
</table>

Sample Details
Laboratory ID: T47707-2
Sample Type: Product
Description: Apple Juice (Mill Orchard)
Date Sampled: 11/02/2015 10:30
Date Received: 12/02/2015 08:20

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<tbody>
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<td>In House Method</td>
</tr>
<tr>
<td>Glucose*</td>
<td>2.3</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>0.9</td>
<td>g/100g</td>
<td>In House Method</td>
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</tbody>
</table>

Results apply to samples as received

Our routine detection limits for chemical testing relate to samples with a clean matrix.
Reported detection limits may be higher for individual samples if there is insufficient sample or the matrix is complex.

< means less than, > means greater than

Date Generated: 24/2/15

Authorised by: Joshua Fitzgerald
Position: Laboratory Technician, Food Laboratory
Signature:

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* Indicates an analysis that is not IANZ accredited

Report Number: 568100
Project Number: T47707
V17.59
SLF
Certificate of Analysis: Final

Project Number: T47707

University of Otago
PO Box 56
DUNEDIN

Attention: Sara White

Customer Order No: 9829FM
Email Recipients: Sara White

Sample Details
Laboratory ID: T47707-1
Description: Whole Raw Apples x 5 (Royal Gala)
Sample Type: Product
Date Sampled: 11/02/2015 10:30
Date Received: 12/02/2015 08:20

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Sample Details
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Reported detection limits may be higher for individual samples if there is insufficient sample or the matrix is complex.

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Date Generated: 24/2/15

Authorised by: Joshua Fitzgerald
Position: Laboratory Technician, Food Laboratory
Signature: [Signature]

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* Indicates an analysis that is not IANZ accredited
Authorised by: Paul Parker
Position: Section Head, Food Laboratory
Signature: [signature image]
1. **PRINCIPLE OF METHOD**

1.1. A sample of food is extracted with 30% acetonitrile (ACN), the protein precipitated, the liquid clarified and injected into an ultraperformance liquid chromatography (UPLC) system with evaporative light scattering detection (ELSD).

2. **REFERENCES**

2.1. Waters Corporation Technical Note: Recommended conditions for separating carbohydrates using Acquity UPLC BEH Amide columns; WAT64250-21111.

2.2. HPLC in Food Analysis; R. MacRaeved; Academic Press, 1982.


---

**RECOMMENDED CONDITIONS FOR SEPARATING CARBOHYDRATES USING ACQUITY UPLC BEH AMIDE COLUMNS**

Based upon your selection, these ACQUITY UPLC conditions are recommended.

**LC CONDITIONS**

- **Detector:** ACQUITY ELSD
- **Detector Settings:** Data Rate: 10 pps
  - Time Constant: Normal
  - Gain: 200
  - Pressure: 40 psi
  - Drift Tube: 40 °C
  - Nebulizer: Cooling
  - Data Processing: Savitsky-Golay smoothing (level 17)
  - Mobile phase A2: 100% acetonitrile (to flush column after use)

**Columns:** ACQUITY UPLC BEH Amide 2.1 x 100 mm, 1.7 μm

**Part Number:** 186004001

**Mobile Phase A:** 80:20 acetonitrile with 0.2% TEA

**Mobile Phase B:** 30:70 acetonitrile with 0.2% TEA

**Mix:** 90% A1:10% B1 (75% acetonitrile with 0.2% TEA)

**Flow Rate:** 0.13 ml/min

**Run Time:** 12 min

**Standard Samples:** Fructose, glucose, sucrose, maltose, and lactose

**Temperature:** 35 °C

**Injection:** 1.3 μl (5 μl loop with partial needle overfill)

---

Fructose  
Sucrose  
Glucose  
Maltose  
Lactose

---

P: PMU

1. Fructose  
2. Glucose  
3. Sucrose  
4. Maltose  
5. Lactose

---

Analyze each 1 mg/ml in 50:50 ACN/H2O

---

WAT64250 — 21111

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34 Maple Street Milford, MA 01757 U.S.A. T: 1 508 478 2000 F: 1 508 872 1990

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Appendix D
1.29 Communication strategy:

Two weeks before:

Black board: Have Information sheet, lab timetable and ethical consent form available on Black board.

One week before:

Email from Andrew once students are enrolled and streamed: – lab streams, information sheet, pre-lab instructions and contact details if they have a time table clash.

Day before first test session:

In lectures with Bernard Venn, Thursday before first test session: verbal reminder to attend and pre-lab instructions.

Day of test session:

At test session: Ethics consent form, information sheet and fill-in form for self-reported baseline details.
Appendix E
1.30 Blood Collection protocol

1. Greet participant
2. Explain that you’ll be taking a sample and ask them if you need their cooperation to encourage blood flow, drop hand down, etc.
3. Ensure you use one of the middle three fingers that have had a wheat bag applied.
4. Alcohol swab the finger.
5. Prick with lancet and use the square gauze wipe to quickly clear away the initial blood, to prevent alcohol dilution of blood.
6. Collect sample into BD Microtainers – aim for at least 375 μL (halfway between 250 μL and 500 μL marker).
7. Apply the very tip of micro cuvette to a drop of blood. Wipe the outside of the micro cuvette with the square gauze wipe and place in the HemoCue glucose 201+ unit.
8. Invert mini tube with the lid on 7-9 times so that the blood mixes with the K₂EDTA anti-coagulant coating inside the tube.
9. Give participant cotton swab or bandaid and ask them to apply pressure.
10. Place BD Microtainers for uric acid assay in chilli-bin
11. Ensure participant records the blood glucose concentration on their data collection form.

Additional notes:

Use saline to clean the area to minimise clotting.

Prevent squeezing finger hard, as you will dilute the blood sample with interstitial fluid.
**Information Collection Sheet: Week 2**

Student ID: ____________________________

Contact phone number: ____________________________

In the last two weeks have you made major changes to your dietary or physical activity habits? E.g. purposely decreased portion sizes, eliminated or added a food group or drastically increased the amount of time you spend exercising? ____________ YES/NO

- If yes, please tell us what you have changed:

Have you eaten anything from 10pm last night? ________________ YES/NO

If yes, what have you eaten since 10pm last night, and when?

Did you have a carbohydrate rich meal for dinner last night? ________ YES/NO

Did you drink alcohol yesterday or this morning? ________________ YES/NO

If yes, how many standards did you consume? _____

Did you do any physical activity outside of your normal routine yesterday or this morning? _____________________________________________ YES/NO

If yes, what did you do?

Have you started any new medications or supplements since your last lab? ___ YES/NO

If yes, what type and frequency?

**Blood Glucose Readings- Week 2**

You will have 3 blood glucose tests done, please record the readings below;

Baseline:

30 minutes:

60 minutes:

Thank you very much for participating in this research! We appreciate your time and effort, please contact your course coordinator Dr Bernard Venn if you have any questions.
Satiety Questionnaire

Time: 30 minutes

This questionnaire is to measure the intensity of satiety before test meal consumption. Please indicate by marking a line along the scale to represent the level of satiety you are currently experiencing.

How hungry do you feel?

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

How satisfied do you feel?

I cannot eat another bite   I am completely empty

How full do you feel?

Not at all full   Totally full

How much do you think you can eat?

Nothing at all   A lot

How comfortable do you feel?

Very uncomfortable   Very comfortable
Gastrointestinal wellbeing

**Time:** 30 minutes

This questionnaire is to measure the intensity and type of any gastrointestinal symptoms experienced by participants’ post-test meal consumption. Please indicate by marking the tick box you identify with, that represents the severity of any gastrointestinal symptoms you have experienced.

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<tr>
<th>Did you experience during the last 30 minutes</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Quite a lot</th>
<th>Severe</th>
<th>Very severe</th>
<th>Unbearable</th>
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</thead>
<tbody>
<tr>
<td>Abdominal rumbling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix H

Dilution curve

UA2 test kit reagent
1.31 Dilution curve

Figure 8. The correlation between uric acid concentration and the percentage of control Path was determined to be linearly distributed.
The color intensity of the quinone-dimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Reagents - working solutions
R1 Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L, fatty alcohol polyglycol ether: 4.8 %; ascorbate oxidase (EC 1.10.3.3, zinc) ≥ 83.5 μkat/L (25 °C); stabilizers
R2 Phosphate buffer: 0.1 mol/L, pH 7.8; potassium hexacyanoferrate (II): 0.3 mmol/L; 4-aminophenone ≥ 3 mmol/L; uricase (EC 1.7.3.3, Arthrober sp 2407) ≥ 83.4 μkat/L (25 °C) peroxidase (PDD) (EC 1.11.1.7; horseradish) ≥ 50 μkat/L (25 °C); stabilizers

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
UA2 Shelf life at 2-8 °C: See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer: 8 weeks
Diluent NaCl 9 % Shelf life at 2-8 °C: See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer: 12 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.
Serum: Plasma: U-heparin and K₂-EDTA plasma
EDTA plasma values are approximately 7 % lower than serum values.

To prevent ureate precipitation in urine samples, add sodium hydroxide to keep urine alkaline (pH > 8.0). To achieve stated uric acid stability, add NaCl to prior to sample collection. Urine samples are diluted 1 + 10 with distilled/deionized water or 0.9 % NaCl. This dilution is taken into account in the calculation of the results.

Centrifuge samples containing precipitates before performing the assay.
UA2
Uic Acid ver.2

Stability in serum/plasma: 15
5 days at 2-8 °C
6 months at (15)-25 °C

Stability in urine: 15
(upon NaOH addition):
4 days at 15-25 °C

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
General laboratory equipment

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

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</tr>
<tr>
<td>Reaction time / Assay points</td>
<td>10 / 23-27</td>
</tr>
<tr>
<td>Wavelength (sub/main)</td>
<td>700/546 nm</td>
</tr>
<tr>
<td>Reaction direction</td>
<td>Increase</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dL (μmol/L, mg/L)</td>
</tr>
<tr>
<td>Reagent pipetting</td>
<td>Diluent (H2O)</td>
</tr>
<tr>
<td>R1</td>
<td>72 µL 25 µL</td>
</tr>
<tr>
<td>R3</td>
<td>14 µL 20 µL</td>
</tr>
<tr>
<td>Sample volumes</td>
<td>Sample Sample dilution</td>
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<tr>
<td>Normal</td>
<td>3 µL – –</td>
</tr>
<tr>
<td>Decreased</td>
<td>12 µL 15 µL 135 µL</td>
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<tr>
<td>Increased</td>
<td>6 µL – –</td>
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<td>Reaction direction</td>
<td>Increase</td>
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<td>Sample Sample dilution</td>
</tr>
<tr>
<td>Normal</td>
<td>3 µL – –</td>
</tr>
<tr>
<td>Decreased</td>
<td>3 µL 6 µL 160 µL</td>
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<tr>
<td>Increased</td>
<td>6 µL 15 µL 150 µL</td>
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<td>Sample Sample dilution</td>
</tr>
<tr>
<td>Normal</td>
<td>3 µL – –</td>
</tr>
<tr>
<td>Decreased</td>
<td>3 µL 6 µL 160 µL</td>
</tr>
<tr>
<td>Increased</td>
<td>6 µL 15 µL 150 µL</td>
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<tr>
<td>Increased</td>
<td>6 µL 15 µL 150 µL</td>
</tr>
</tbody>
</table>

Calibration

Calibrators S1: H2O
S2: C.f.a.s.

Calibration mode Linear

Calibration frequency 2-point calibration
- after reagent lot change
- as required following quality control procedures

Traceability: This method has been standardized against IDMS.17

Quality control

Serum/plasma
For quality control, use control materials as listed in the “Order information” section.
Other suitable control material can be used in addition.

Urine Quantitative urine controls are recommended for routine quality control.
The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained shall fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.
Conversion factors: mg/dL x 59.5 = μmol/L
mg/dL x 10 = mg/L
Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a uric acid concentration of 7 mg/dL (417 μmol/L).

Serum/plasma

Lactemia: No significant interference up to an L index of 40 (approximate conjugated and unconjugated bilirubin concentration: 684 μmol/L (40 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 μmol/L (1500 mg/dL)).

Lipemia (Tripalidyl): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ascorbic acid < 0.17 mM (20 μg/mL) does not interfere.

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

Exceptions: Calcium dobesilate causes artificially low uric acid results.

Urines react specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction. In very rare cases, gammapathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.

Urine

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

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

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the Carry over evasion list can be found with the NaOH/SIMS/Multiclear/SCCS or the NaOH/SIMS/SimPCr1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.

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

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

Limits and ranges

Measuring range

Serum/plasma

0.2-25.0 mg/dL (11.9-1487 μmol/L)

Determine samples having higher concentrations via the run function. Dilution of samples via the run function is a 1:2.5 dilution. Results from samples diluted by the run function are automatically multiplied by a factor of 2.5.

Urine

2.2-275 mg/dL (131-16382 μmol/L)

Determine samples having higher concentrations via the run function. Dilution of samples via the run function is a 1:2.5 dilution. Results from samples diluted by the run function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Lower detection limit of the test

Serum/plasma

0.2 mg/dL (11.9 μmol/L)

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

Urine

2.2 mg/dL (131 μmol/L)

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

Expected values

Serum/plasma

Males: 3.4-7.0 mg/dL (202.3-416.5 μmol/L)

Females: 2.4-5.7 mg/dL (142.8-339.2 μmol/L)

Urine (reference range according to Krieg and Colombo)

1st morning urine: 37-92 mg/dL (2200-5475 μmol/L)

24-hour urine: 200-1000 mg/day (1200-5900 μmol/day)

(corresponding to 13-67 mg/dL (773-3986 μmol/L)

(calculated from a urine volume of 1.5 L/24 h)

Urine (reference range according to Tietz)

Average diet

Males: < 400 mg/24 hours

Females: < 400 mg/24 hours

Low purine diet

Males: < 1000 mg/24 hours

Females: < 400 mg/24 hours

High purine diet

Males: < 1000 mg/24 hours

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability* (n = 21), intermediate precision** (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Serum/plasma

Repeatability* Mean SD CV

mg/dL (μmol/L) mg/dL (μmol/L) %

Precnorm U 4.54 (270) 0.04 (2) 0.9

Precpach U 11.1 (660) 0.1 (6) 0.7

Human serum 1 4.03 (240) 0.04 (2) 1.0

Human serum 2 7.23 (430) 0.06 (4) 0.8

Intermediate Mean SD CV

precision** mg/dL (μmol/L) mg/dL (μmol/L) %

Precnorm U 4.47 (266) 0.07 (4) 1.5

Precpach U 11.1 (660) 0.12 (12) 1.6

Human serum 3 3.98 (236) 0.05 (3) 1.3

Human serum 4 2.17 (127) 0.09 (6) 1.3

Urine

Repeatability* Mean SD CV

mg/dL (μmol/L) mg/dL (μmol/L) %

Control level 1 11.7 (686) 0.1 (6) 1.2

Control level 2 21.7 (1291) 0.3 (18) 1.3

Urine 1 28.8 (1714) 0.6 (30) 2.1

Urine 2 32.5 (1934) 0.5 (30) 1.5

Intermediate precision** Mean SD CV

mg/dL (μmol/L) mg/dL (μmol/L) %

Control level 1 11.4 (678) 0.2 (12) 1.9

Control level 2 21.3 (1257) 0.3 (18) 1.6

Urine 3 29.3 (1743) 0.9 (54) 3.0

Urine 4 32.1 (1910) 0.8 (48) 2.3

* repeatability = within-run precision

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

Uric acid values for human serum, plasma and urine obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Serum/plasma
Sample size (n) = 89
Passing/Bablok
\[ y = 0.963x + 0.158 \text{ mg/dL} \]
\[ y = 0.966x + 0.224 \text{ mg/dL} \]
\[ r = 0.969 \]
The sample concentrations were between 2.70 and 23.4 mg/dL (161 and 1592 µmol/L).

Urine
Sample size (n) = 86
Passing/Bablok
\[ y = 0.997x + 0.456 \text{ mg/dL} \]
\[ y = 0.998x + 0.522 \text{ mg/dL} \]
\[ r = 0.999 \]
The sample concentrations were between 6.35 and 269 mg/dL (378 and 16000 µmol/L).

References