Fructose Intake from Sugar-Sweetened Beverages and Acute Plasma Uric Acid Production

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Abstract

**Background:** Sucrose intake, half of which is fructose, has been increasing over recent decades. Additionally, the introduction of high fructose corn syrup in North America in the 1970s has also led to an increase of fructose in the diet. Fructose has caused much debate in the scientific and wider community. The unregulated metabolic pathway of fructose leads to uric acid and fat production, which contributes to the burden of disease. Studies have linked fructose consumption to obesity, diabetes, gout, hypertension, fatty liver disease and increased visceral fat. Some researchers question the previous research, with the largest concern being around the feasibility of the fructose dose used in the current literature, which is producing the pathological change.

**Objective:** To determine the effects of two commonly consumed single-serve amounts of a sugar-sweetened beverage on acute plasma uric acid concentration in the body within a healthy New Zealand population.

**Design:** Randomised crossover control trial

**Methods:** Forty-two participants were randomised to the control group or the sugar-sweetened beverage intervention. Across two testing days, the control group consumed a 600 ml fructose and a 600 ml glucose beverage, while the intervention group consumed a 355 ml and 600 ml Sprite beverage in random order. Control beverages were matched for fructose content with the 600 ml bottle of Sprite (26.7 g). The 355 ml can of Sprite delivered 15.8 g of fructose. Blood samples were collected at baseline, 30 and 60 minutes and were analysed for plasma uric acid. This study design enabled the assessment of the effect of different doses of fructose and the form of fructose, pure or as a component of sucrose on plasma uric acid response.

**Results:** Both sugar-sweetened beverage volumes significantly raised plasma uric acid levels after 30 and 60 minutes (P<0.05). The fructose control produced the largest rise in plasma
uric acid concentration at 60 minutes across all consumed beverages of 26.34 μmol/L (P=0.004). The glucose control significantly decreased plasma uric acid concentration after 60 minutes (P=0.018). IAUC of the plasma uric acid response was measured, with no significant difference in the body’s response between the Sprite beverages (P=0.499) and the Sprite and fructose beverage (P=0.114). However, there was a significant difference in iAUC between the glucose and fructose control (P=<0.001) and the glucose and 600 ml Sprite beverage (P=0.015).

**Conclusion:** Both the 355 ml and 600 ml Sprite exhibited statistically significant rises in plasma uric acid concentration, however, this transient rise in plasma uric acid response was perceived as clinically insignificant. We found no difference in the body’s plasma uric acid response between the pure fructose control and the sucrose sweetened 600 ml Sprite beverage.

**Keywords:** Sugar-sweetened beverage, fructose, plasma uric acid, New Zealand, acute response, gout.
Preface

Emma Carran (candidate) conducted this study under the supervision of Dr. Bernard Venn and PhD Candidate Mr Andrew Reynolds as part of completing a Master of Dietetics. Dr. Bernard Venn was responsible for instigating the research topic, gaining ethical approval, registering the study, conceiving the study design and supervising the thesis write-up. Andrew Reynolds was responsible for conceiving the study design, recruitment organisation, data collection, supervising biochemical assessment and thesis write-up.

The candidate was responsible for:

- Conducting a literature review on the topic of fructose and uric acid production;
- Input into study design, including the amount of fructose that should be tested;
- Participant recruitment; pre-study engagement including verbal, online and written communication with ongoing rapport throughout the trial;
- Liaising with study stakeholders over the formation and collation of this thesis;
- Being an involved member of the study team throughout data collection. This included pretesting beverages, liaising with laboratory staff, purchasing test foods, weighing control sugars, designing and printing laboratory packs and setting up and coordinating the testing days;
- Data collection, collation and entry of the data;
- Conducting the biochemical analysis including, the centrifuging and separation of plasma from the blood samples, quality control and blood glucose and uric acid determination;
- Input into the statistical analysis of the uric acid results conducted by Dr. Jill Haszard;
- Interpreting the results with statistical and clinical consideration;
- Writing and compiling this thesis.

The candidate presented the results of this research in an oral presentation at the Human Nutrition department seminar on May 28th, 2015, before the submission of this thesis.
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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>HFCS</td>
<td>High fructose corn syrup</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>IMP</td>
<td>Inosine monophosphate</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
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<td>GLUT</td>
<td>Glucose transporters</td>
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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
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<tr>
<td>GAP</td>
<td>Glyceraldehyde-3-phosphate</td>
</tr>
<tr>
<td>DHAP</td>
<td>Dihydroxyacetone phosphate</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>NHANES</td>
<td>National health and nutrition examination survey</td>
</tr>
<tr>
<td>mg/Dl</td>
<td>Milligram/decilitre</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>oz</td>
<td>Ounces</td>
</tr>
<tr>
<td>μmol/L</td>
<td>Micromole/litre</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>MD</td>
<td>Mean difference</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>MDiet</td>
<td>Master of Dietetics</td>
</tr>
<tr>
<td>ELC</td>
<td>Emma Louise Carran</td>
</tr>
<tr>
<td>μL</td>
<td>Microlitres</td>
</tr>
<tr>
<td>PNU</td>
<td>Precinorm U</td>
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<tr>
<td>PPU</td>
<td>Precipath U</td>
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<tr>
<td>iAUC</td>
<td>Incremental area under the curve</td>
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1 Introduction

Fructose is a sugar that occurs naturally in fruit in its free form, or joined to glucose in the disaccharide sucrose. Fructose can be added to foods as a sweetener and is a major component of high fructose corn syrup (HFCS). In New Zealand, sucrose from cane sugar is the main source of added sugar in processed foods. Along with free fructose in ripe fruit, sucrose is the main source of fructose in the New Zealand diet \(^{(1)}\). HFCS is not widely used in New Zealand and is most commonly found in imported products \(^{(1)}\).

Researchers have linked the rise in the worldwide consumption of fructose since the 1970s with the rise of food processing and use of HFCS and sucrose as commercial sweeteners \(^{(1, 2)}\). With increased consumption of foods that contain added sugars, concern has been raised about the fructose component of these sweeteners and the amount of excess energy such foods provide. Some cross-sectional cohort studies have linked rising fructose consumption to the increased prevalence of obesity, cardiovascular disease, hypertension, diabetes and gout \(^{(3-7)}\). Studies have identified sweetened beverages as a major contributor to the increasing fructose consumption, with HFCS-sweetened beverages being found to increase serum uric acid concentrations more than sucrose-sweetened beverages \(^{(8)}\).

In New Zealand, gout is particularly significant due to the prevalence and impact of the disease particularly in Māori and Pacific Islander populations \(^{(9)}\). Gout is caused by excess uric acid leading to the formation of monosodium urate crystals in and around the joints \(^{(10)}\). The metabolism of fructose by the liver is the only sugar digestion process identified to increase the production of uric acid \(^{(11)}\). Fructose metabolism is unregulated and ultimately results in the conversion of adenosine triphosphate (ATP) to inosine mono phosphate (IMP), activating the catabolic pathway leading to uric acid production \(^{(11)}\).

The World Health Organization (WHO) recently released guidelines recommending the daily intake of free sugars should be below 5% of overall energy intake \(^{(12)}\). The National Adult
Nutrition Survey 2008/2009 determined that in New Zealand approximately 8-10% of daily energy comes from sugars, with adolescents consuming greater amounts\(^{(12, 13)}\). Over 50% of fructose intake in the New Zealand diet comes from non-fruit sources such as sweetened and alcoholic beverages, confectionery, convenience foods and baked goods\(^{(13)}\). Sugar-sweetened beverages make up 18% of daily fructose intake\(^{(13)}\).

The conflicting evidence on the effects of fructose intake has sparked global debate\(^{(14, 15)}\). Some argue that demonising fructose is unwarranted and that negative health effects are being observed due to the excess energy provided by added sugars\(^{(14, 16-18)}\). Furthermore, the European Food Safety Authority has released a public health claim, stating that substituting fructose for glucose and sucrose in foods has a positive effect on postprandial glycaemia\(^{(19)}\). Other scientists maintain that the introduction of HFCS and the increased proportion of fructose in our diets have caused the increased prevalence of negative health outcomes and non-communicable diseases\(^{(3, 15, 20-23)}\).

Prospective cohort studies have been conducted across various age groups, observing a positive relationship between fructose intake and serum uric acid concentrations though these studies are unable to determine causality\(^{(4, 7, 24)}\). An intervention study using 150 g/day of fructose found a significant increase in visceral adiposity, compared with those consuming glucose\(^{(21)}\). This amount of fructose far exceeds the average 43-53 g consumed daily in New Zealand, with other studies also testing similar amounts of fructose\(^{(16, 25, 26)}\). A meta-analysis suggests that many long-term adverse effects, such as increased body weight and fasting lipid concentrations, are not seen until fructose doses exceed 100 g/day\(^{(27)}\), although researchers observed a statistically significant rise in acute serum uric acid after an acute 39 g fructose dose\(^{(8)}\).

The purpose of the current study is to determine the acute plasma uric acid response from fructose after consuming two readily available serving sizes of a commercial sugar-sweetened beverage.
2 Literature Review

2.1 Methodology of this Literature Review
This literature review focuses on the relationship between oral fructose intake and acute postprandial serum uric acid concentrations and the potential health impact on gout.

This literature review aims to:

1. Provide an overview of fructose, high fructose corn syrup, fructose biochemistry and fructose metabolism;
2. Deliver an overview of uric acid and its association with gout;
3. Discuss the current research around fructose consumption, serum uric acid concentrations and gout;
4. Examine the epidemiology of fructose intake and gout in the New Zealand context;
5. Determine gaps that exist in the research around fructose intake and serum uric acid concentrations;

Literature was retrieved from the following databases: Medline via Ovid, Embase and PubMed. Keywords included were ‘fructose’, ‘uric acid’, ‘gout’, ‘New Zealand’, ‘sweetened beverages’, ‘hyperuricemia’, and ‘dose response’. Further literature was identified using the reference lists of the published articles that were collected. Literature was deemed to be appropriate if written in English and had human participants.

2.2 Fructose

2.2.1 What is Fructose?
Fructose is a sweet tasting, monosaccharide carbohydrate molecule with a furanose ring structure \(^{8,11,28}\). Fructose often pairs with a glucose molecule and together they form a
disaccharide called sucrose (Figure 2.1) \(^{(29)}\). Sucrose is otherwise known as table sugar, which is most commonly derived from sugar cane or beets \(^{(29, 30)}\).

![Chemical structure of Glucose, Fructose and Sucrose](image)

**Figure 2.1** Chemical structure of Glucose, Fructose and Sucrose \(^{(11)}\)

Fructose may be present in food as free fructose or bound as a component of naturally occurring sucrose; these are intrinsic sugars \(^{(29)}\). Fructose may also be added to food, primarily through the use of sucrose, but also through high fructose corn syrup in North America, and to a lesser extent as free fructose \(^{(20, 29)}\). Intrinsic fructose is in foods such as fruits, vegetables and pure fruit juice \(^{(29)}\). Added fructose is mainly found in the home in the form of table sugar and corn syrup \(^{(20, 29)}\). It is used as an ingredient in many manufactured products, including but not limited to sweetened beverages, baked goods, refined products and canned fruits \(^{(20, 29)}\).

### 2.2.2 Sweeteners

The major sources of dietary fructose are sucrose or HFCS \(^{(15, 31)}\). In the United States of America (USA), fructose is estimated to provide around 10.2% of daily energy intakes, with some individuals, particularly adolescents, consuming more than 15% of total energy \(^{(15)}\). Fruits and vegetables provide approximately one-quarter of this fructose, with the balance coming from added fructose found in sweetened beverages, sugar and HFCS commercial products \(^{(15)}\).
HFCS use has risen since the 1970s, particularly in the US market, contributing to over 40% of added sweeteners in food \(^{(20)}\). HFCS is preferred over sucrose as it is inexpensive to manufacture, making it more profitable \(^{(20)}\). It also contains a higher amount of fructose, which has a higher sweetness rating than sucrose \(^{(20)}\). Sucrose is comprised of 50% glucose and 50% fructose. HFCS comes in two different forms; HFCS-55, which consists of 55% fructose and 42% glucose and is primarily used to sweeten beverages. The second form is HFCS-42, which consists of 42% fructose and 53% glucose and is primarily used to sweeten products such as baked goods and confectionery \(^{(2)}\).

In New Zealand, the primary sweetener used is sucrose, however, many imported products, particularly from North America, contain HFCS \(^{(1)}\). In New Zealand adults, sucrose consumption is estimated to provide 8-10% of daily energy, with 128 g and 103 g of total sugars being consumed daily by males and females respectively \(^{(13,32)}\). Fructose consumption, including free fructose and half of the sucrose intake is on average 53 g/day and 43 g/day for New Zealand males and females respectively. Approximately 42% of this comes from fruits and vegetables while the rest comes largely from sweetened and alcoholic beverages, confectionary and baked goods \(^{(13)}\). Fructose intake from sweetened beverages is higher in the younger age groups, particularly 15-30-year-old males, and 15-18-year-old females. These age groups consume 32% of their dietary fructose from sugar-sweetened beverages \(^{(13)}\).

### 2.2.3 Absorption and Utilisation of Fructose and Glucose

The pathways that metabolise fructose and glucose differ in key ways. Upon entering the liver, fructose is phosphorylated via fructokinase, whereas the phosphorylation of glucose occurs via hexokinase. From there fructose and glucose undergo different metabolic pathways. The concerning differences are that the metabolism of fructose is unregulated meaning there is no metabolic process that controls the amount and speed of fructose
metabolism. Additionally, fructose is the only sugar which when metabolised results in the production of uric acid \(^{(24, 33)}\).

Glucose and fructose can enter the body either in free form or as part of sucrose. If bound in sucrose, sucrose is rapidly hydrolysed into fructose and glucose monosaccharides via sucrase in the small intestine. Transportation of these monosaccharides occurs across the intestinal wall of the small intestine via GLUT transporters \(^{(34)}\). Once in the blood stream, the majority of fructose travels to the liver to be metabolised \(^{(11)}\). Some fructose enters the systemic circulation and this may be metabolised in the muscle, although the amount is small in comparison to that handled by the liver \(^{(11)}\). When metabolised by the liver, fructose bypasses the first two highly regulated steps of glycolysis. Instead, fructose is metabolised to fructose-1-phosphate by fructokinase \(^{(11, 30)}\). This step requires ATP and leads to the degradation of ATP to adenosine diphosphate (ADP) \(^{(11)}\).

![Figure 2.2 Metabolic pathway of Fructose to Uric Acid \(^{(24)}\)](image-url)
Glucose is also phosphorylated and uses ATP. However, it does not produce uric acid. The metabolic basis for this difference is the extremely fast rate of fructose phosphorylation, whereas glucose metabolism is more tightly regulated through the rate limiting enzyme phosphofructokinase (35). After the first step of hepatic fructose metabolism, fructose-1-phosphate traps inorganic phosphate and intracellular levels decrease. As a result, intracellular ADP is converted to adenosine monophosphate (AMP). AMP is then converted to IMP via the enzyme myoadenylate deaminase, causing IMP levels to elevate. Subsequently the elevated AMP and IMP activate the catabolic pathway, which leads to increased uric acid production (Figure 2.2) (24, 36).

Conversion of fructose-1-phosphate to glyceraldehyde can go one of two ways, both ending in the production of the glycolytic intermediate glyceraldehyde-3-phosphate (GAP). The first is a simple reaction in which the conversion of glyceraldehyde to GAP occurs via glyceraldehyde kinase.

![Figure 2.3 Metabolic pathway of Fructose to Glucose](image)

1 = hexokinase, 2 = phosphoglucoisomerase, 3 = hexokinase, 4 = fructokinase, 5 = glycogen phosphorylase, 6 = phosphofructokinase, 7 = aldolase, 8 = triose phosphate isomerase, 9 = triose kinase, 10 = several enzymes including pyruvate kinase, 11 = pyruvate dehydrogenase complex.
The second requires four enzymes to get from glyceraldehyde to GAP via the glycolytic intermediate dihydroxyacetone phosphate (DHAP) \(^{(11)}\). Both reactions require ATP and the oxidation and reduction of NADH. GAP is a glycolytic intermediate and enters into glycolysis where it can be converted into pyruvate or go through gluconeogenesis and be converted to glucose (Figure 2.3) \(^{(11, 34)}\).

The metabolism of fructose has led to the hypothesis that excess fructose consumption is linked to an increased incidence of obesity \(^{(11)}\). The metabolic pathway of fructose suggests that when excess fructose is consumed, fructose catabolism in the liver bypasses the phosphofructokinase (enzyme 6 in figure 2.3) step in glycolysis. This enzyme converts F-6-P to fructose-1,6-bis-phosphate. By avoiding this rate-limiting step, fructose metabolism avoids being regulated and F-6-P is converted straight into GAP, which can be converted into glucose. This disrupts fuel metabolism and increases the rate of molecules passing through glycolysis. The molecules are then directed towards lipid synthesis in the absence of the demand for ATP production, leading to fat production in the body \(^{(11)}\).

### 2.2.4 Proposed Health Impact of Fructose Consumption

Fructose has come under scrutiny since the use of HFCS in food processing rose in the 1970s \(^{(3, 37)}\). Arguments around the rising fructose consumption and proposed adverse health effects came forth in the 1980s. It was hypothesised that fructose altered the metabolic pathways of glucose, uric acid, copper and lipids and was a possible causal factor for hypertension and cardiovascular disease (CVD) \(^{(5, 37-39)}\). Since then studies have been conducted to investigate the effects of fructose consumption on health outcomes; including blood pressure, CVD, obesity, BMI, diabetes and metabolic syndrome \(^{(3-5, 8)}\). Many of these studies have found a significant association between fructose and the aforementioned health outcomes \(^{(3, 4, 6, 8)}\).
Using a randomised control study, Stanhope et al. 2009 looked at the association between fructose intake and visceral adiposity, lipids and insulin sensitivity. Participants were given either glucose or fructose-sweetened beverages that provided 15% of their daily energy intake for eight weeks. This study provided supporting evidence for the fructose hypothesis. It concluded that dietary fructose consumed under the study conditions was a causal factor in developing dyslipidemia, decreased insulin sensitivity and increased visceral adiposity in overweight/obese adults (21).

It is important to look at the dose of fructose used when analysing fructose studies (40). A meta-analysis conducted by Livesey et al. investigated the doses used in intervention studies against various health outcomes (27). This meta-analysis found that fructose intakes from 0-90 g/day had a beneficial effect on HbA1C levels. There was no significant effect on postprandial triacylglycerol when fructose intake was <50 g/day. There was no significant effect on fasting plasma triacylglycerol with fructose intakes of up to 100 g/day, although there was a significant dose-response effect with fructose intakes between 100 g/day and 350 g/day. There was no significant effect observed on body weight with fructose intakes <100 g/day in adults. A significant short-term increase in body weight was not observed until a 213 g/day intake of fructose was administered (27).

In the US, more than 95% of adults consume <100 g/day of fructose from all sources. On average, adolescent males have the largest fructose intakes with approximately 20% consuming upward of 150 g/day (27, 40). This suggests that results of many studies conducted using >100 g/day would be of little relevance to many adults, particularly females, and would be of most relevance to adolescent males (27, 40). These results would also be of little relevance to the majority of New Zealanders, where the average fructose intake is average 53 g/day and 43 g/day for males and females respectively (13).

Some researchers oppose the view that fructose is linked to and is responsible for many adverse health effects. They claim that methodological flaws in studies surrounding fructose
dose used, and the conditions that the fructose was consumed under (not as part of a food) does not reflect normal intake and therefore is not applicable to the wider population (14, 37). Sievenpiper et al. and Ha et al. conducted meta-analyses examining the claims linking fructose consumption to increased body weight and hypertension (17, 18). They looked at controlled feeding trials where fructose was iso calorically substituted for other carbohydrates. Examining blood pressure, Ha et al. found that when isocalorically exchanged, fructose significantly decreased diastolic and mean arterial blood pressure and had no effect on systolic blood pressure (17). For body weight, Sievenpiper et al. found no significant difference in body weight when consuming fructose isocalorically (95% CI, -0.37, 0.10). Significance was only observed when fructose was consumed hyper calorically (95% CI, 0.26, 0.79) (18).

2.3 Uric Acid and Gout

2.3.1 Uric Acid

Production of uric acid is a result of purine nucleotide catabolism (11, 41). Once uric acid is produced it dissolves into the bloodstream and travels to the renal system, to be filtered by the kidney and expelled in the urine (42).

2.3.2 Hyperuricemia and Gout

When the body produces too much uric acid, the kidney is unable to filter and expel excess uric acid (42). Serum uric acid then remains high and this is referred to as hyperuricemia (42). Hyperuricemia is the precursor of gout and is also associated with other adverse health outcomes, including CVD and diabetes (43). Gout is extremely painful arthritic joint inflammation that often has a very sudden onset (11). It occurs when the body fluids become saturated with uric acid, causing the formation of monosodium urate crystals in and around the joints (10). Gout is one of the most prevalent
inflammatory diseases in New Zealand, particularly among men and is associated with a decreased quality of life \textsuperscript{(10, 43, 44)}. The incidence of gout has risen over recent years, with common hypotheses for this being changes in diet, lifestyle and the rise of obesity \textsuperscript{(45-47)}.

2.3.3 Measuring Uric Acid

Uric acid can be measured in urine and blood serum. However, it is the serum uric acid concentration that is of most relevance to adverse health effects as this indicates how much uric acid remains in the body. The reference range for fasting serum uric acid concentration is between 3.5 mg/dL and 7.2 mg/dL \textsuperscript{(42)}. Using NHANES III laboratory definition, hyperuricemia is indicated if serum uric acid concentration is $>7.0$ mg/dL (619 μmol/L) for men and $>5.7$ mg/dL (504 μmol/L) for women \textsuperscript{(43)}.

2.3.4 Genetic Factors Relating to Gout

SLC2A9 is a human gene, which is a high-capacity urate transporter \textsuperscript{(26)}. Variants have been identified within SLC2A9 that exchange uric acid for fructose and glucose, that have been found to influence serum uric acid concentration and excretion \textsuperscript{(1, 48)}. These variants result in a lower uric acid excretion and subsequently increase the risk of high serum uric acid, hyperuricemia and gout \textsuperscript{(26)}.

2.3.5 Dietary Factors and Hyperuricemia

Many foods have been determined to cause and exacerbate hyperuricemia and gout \textsuperscript{(10)}. Purine rich foods such as meats and seafood, alcoholic beverages particularly beer, and fructose and sugar-sweetened beverages have been identified as dietary risk factors for hyperuricemia and gout \textsuperscript{(10, 44)}. Vitamin C, dairy products and coffee are thought to have a positive effect, reducing serum uric acid concentration \textsuperscript{(10, 44)}. Vitamin C, coffee and dairy products are believed to exert uricosuric effects and enhance urate renal excretion \textsuperscript{(10, 49)}. The caffeine in
coffee also acts as an inhibitor of xanthine oxidase, a major enzyme in purine metabolism and, therefore, decreases the generation of uric acid \(^{(10)}\).

### 2.4 Impact of Fructose Consumption on Gout

#### 2.4.1 Fructose Consumption and Gout

The prevalence and incidence of gout have increased in the US in the past 30 years, coinciding with the considerable increase in HFCS and sugar-sweetened beverage consumption \(^{(7)}\). Fructose is the only sugar that is known to have an impact on serum uric acid concentration in the body \(^{(36)}\). It has been feasibly hypothesised that the increase in sugary beverage consumption has contributed to the rise in many adverse health outcomes, including hyperuricemia and gout \(^{(36)}\).

Two large prospective cohort studies, one in men and one in women, were conducted by Choi et al. to assess whether a relationship exists between fructose consumption and risk of gout \(^{(7, 24)}\). The first was published in 2008 and looked at the link between fructose and risk of gout in men \(^{(7)}\). This cohort spanned over 12 years and used data from the health professional follow-up study. The study consisted of 46,393 males and data on fructose consumption from soft drinks was collected based on servings \(^{(7)}\). Their findings suggest that compared to those consuming less than one serving of sweetened beverage per month the multivariate relative risk for gout for two or more servings per day was 1.85, one serving per day was 1.45, and for 5-6 servings of sugar-sweetened beverages a week was 1.29 \(^{(7)}\).

The second study conducted in women produced a similar trend \(^{(24)}\). This study spanned over 22 years and the data from the Nurses’ Health Study was used. Participants included 78,906 women with no history of gout at baseline. Compared to those consuming less than one serving of soft drink per month, the multivariate relative risk for gout risk in women when consuming 1 serving per day was 1.74 and for two or more servings per day was 2.39 \(^{(24)}\).
These studies concluded that increased sweetened beverage intake was significantly associated with increased risk of gout in both men and women (7, 24).

2.4.2 Dose-Response Relationship Between Fructose Intake and Uric Acid Production

Literature has shown that there is a clear association between fructose intake and raised serum uric acid concentrations, but the fructose amount used in the studies has varied. The consensus in the literature is that there is a positive correlation between fructose intake and serum uric acid concentrations (2-4, 6, 30, 36).

Choi et al. used data from the Third National Health and Nutrition Examination Survey (NHANES), 1988-1994 and observed a relationship between sugar-sweetened soft drink intake and serum uric acid concentrations (36). This study was a cross-sectional cohort with 14 671 adolescent participants. In the overall cohort, sugar-sweetened beverage intake was measured based on the category of servings per day of <0.5, 0.5-0.9, 1-3 and >4. Compared with no intake of sugar-sweetened beverages, the odd’s ratios (OR) of developing hyperuricemia were 1.01, 1.34, 1.51 and 1.82 for the respective intakes (P for trend = 0.003) (36).

Nguyen et al. investigated the association between the dose-response consumption of sugar-sweetened beverages and uric acid concentrations in adolescents. This study was a cross-sectional cohort using data from the NHANES 1999-2004, using 4867 adolescents aged 12 to 18 years of age (3). This study used the following intake measurements; 0 oz/day, 1-12 oz/day, 13-24 oz/day, 25-36 oz/day and >36 oz/day. There was a 0.18 mg/dL (15.9 μmol/L) significant increase (P for trend = 0.01) in serum uric acid concentrations between the lowest and highest consumption bracket (3). A limitation of this study was that only one 24-hour diet recall was obtained. Assessing methods of dietary intake, 24-hour recalls are often
confounded by inaccurate recollection and they only give a dietary assessment for one day, therefore don’t assess usual intake \(^{(50)}\).

A cross-sectional study was conducted in 2727 adolescents, measuring consumption of beverages sweetened with HFCS and the association with uric acid \(^{(4)}\). Participants completed a questionnaire and the beverage intakes measured were; non-intake, 1-350 ml, 351-500 ml, 501-750 ml and >750 ml per day. Blood samples were taken three weeks after data collection. Lin et al. found that compared to those who were non-drinkers, all those who consumed the HFCS beverages had higher serum uric acid \(^{(4)}\). Lin et al. also looked into the amount of fructose that was deemed to have the largest effect. There were no significant differences between the >500 ml and >750 ml intake groups. This showed that the effect on serum uric acid was sustained across the >500 ml and >750 ml intakes \(^{(4)}\). It was observed that the 647 participants that consumed >500 ml of a HFCS-sweetened beverage a day had a remarkably higher likelihood of having hyperuricemia (BMI adjusted OR = 2.0-2.1) and had a 0.42 mg/dL (37.13 μmol/L) higher serum uric acid level \(^{(4)}\).

These cohort studies conducted by Choi et al., Lin et al. and Nguyen et al. found similar trends regardless of the age group being tested. Adolescents and adults all displayed a significant increase in serum uric acid concentrations and risk of hyperuricemia as their fructose intake increased \(^{(3, 4, 36)}\). However, a limitation of cohort studies is that they show trends over a period of time and can determine an association, but are unable to determine causality.

The differences between the effects of sucrose and HFCS on uric acid production are still being debated. HFCS is thought to be far more detrimental to health than sucrose. Akhavan et al conducted a randomised control trial in 31 males, 18-35 years of age with a BMI of 20-26 and were non-smokers. This study measured fructose intake and acute uric acid production and were one of the few to do so. The difference in plasma uric acid concentrations after consuming sucrose, HFCS and varying glucose-to-fructose ratio beverages, was measured to
determine if there are significant differences between the sweetener ratios, HFCS and sucrose \(^{(51)}\). Acute plasma uric acid was measured at baseline, 30, 45 and 75 minutes post ingestion. The study found that plasma uric acid concentrations were significantly higher in the higher fructose beverage (F80: G20) compared to those with higher glucose (F20: G80) \(^{(51)}\).

Additionally, there were no significant short-term differences between sucrose, HFCS and G50: F50 beverages on plasma uric acid concentrations \(^{(51)}\). This study determined that there was no significant difference between HFCS-55 and sucrose beverages, which are the two most common added sugars in food processing. As this study involved only male participants, it is unknown if the same effects are seen in females.

Another randomised crossover design study was conducted in 69 male and female adults of varying ethnicities looking at the difference between high fructose and high sucrose sweetened drinks \(^{(8)}\). This study chose a commercially available sugar-sweetened beverage, which was supplied in two forms; the HFCS version contained 39.2 g of fructose and the sucrose-sweetened version contained 34.6 g of fructose \(^{(8)}\). The drinks were both 24 ounces (710 ml) but the HFCS drink had a 13% higher dose of fructose. The study found that there was a significantly higher effect on postprandial serum uric acid concentrations from the HFCS-sweetened beverage than the sucrose-sweetened beverage \((P = 0.0042)\) \(^{(8)}\).

The amount of fructose being administered during trials is a point of importance. A parallel study conducted by Cox et al. compared the effect of prolonged exposure to high fructose and glucose intake \(^{(25)}\). Thirty-two participants were allocated to either a 10-week fructose or glucose diet, where the fructose or glucose comprised of 25% of daily energy intake \(^{(25)}\). The results added to the body of experimental literature that supports that fructose has a more detrimental effect on serum uric acid concentrations than glucose. Both fasting plasma uric acid and 24-hour serum uric acid concentrations were raised significantly between baseline and at the end of the 10-week fructose intervention \(^{(25)}\). There was no significant difference in plasma uric acid levels in the glucose intervention \(^{(25)}\). In the fructose intervention, fasting
plasma uric acid was increased by 0.82 mg/dL (72.49 μmol/L) \((P < 0.0001)\) and 24 hour serum uric acid concentrations were increased by 0.42 mg/dL (37.13 μmol/L) \((P<0.0001)\) \((25)\). This study investigated fructose at 25\% of daily energy intake, which is much higher than the proposed average total sugar intake in New Zealand which is 8-10\% \((13)\).

Wang et al. conducted a systematic review and meta-analysis investigating the effect of fructose feeding under isocaloric conditions \((16)\). Analyses included 21 controlled feeding trials where fructose was either isocalorically exchanged with other carbohydrates or under hypercaloric conditions where a control diet was supplemented with excess energy from fructose (+35\% energy) \((16)\). Under isocaloric conditions there was no significant effect on uric acid in pre-diabetic/diabetic participants \(\text{MD} = -4.09 \, \mu\text{mol/L}\) and in participants without diabetes \(\text{MD} = 1.28 \, \mu\text{mol/L}\) \((16)\). Under hypercaloric conditions, the 35\% excess fructose produced a significant increase in serum uric acid concentrations \(\text{MD} = 31.0 \, \mu\text{mol/L}\) \((16)\). The important factor to note regarding the hypercaloric trials is that 35\% of excess energy exposed the participants to more than double the 95\% percentile (87 g/day) of fructose intake in the US \((14, 16)\).

### 2.5 Rationale for Research

Fructose is a large component of the Western diet and fructose consumption has increased since the 1970s, coinciding with the growing obesity epidemic, the overall increase in daily energy intake, and a decline in physical activity \((20, 21)\). Additionally, the confounding of excess energy in fructose rich diets must be considered when analysing previous literature.

In the New Zealand context the prevalence of gout, particularly in Māori and Pacific Island communities, is well documented \((52)\). An overall national prevalence of gout in New Zealand has been established as 2.69\%, with a higher prevalence of 3.75\% in those greater than 20 years of age \((9)\). Data from the Aotearoa New Zealand Health Tracker observed that gout is more common in Māori and Pacific people \((\text{relative risk (RR) 3.11 and 3.59, respectively.})\)
This indicates that Māori and Pacific people are 3-4 times more likely to develop gout than New Zealand Europeans. Men also have an increased risk (RR 3.58), along with those living in low socioeconomic areas (RR 1.41) and those >65 years of age (RR >40) \(^{(9)}\). The prevalence of gout in elderly Māori and Pacific men is particularly high at >25% \(^{(9)}\). The high prevalence of gout in Māori and Pacific is of particular concern, as poorly controlled gout is associated with increased health care costs, increased disability and decreased quality of life \(^{(52)}\).

There has been a considerable amount of research conducted showing a positive correlation between fructose consumption and various health outcomes. Many studies conducted around fructose have a focus on CVD, obesity, blood pressure or metabolic syndrome without uric acid as an outcome \(^{(53-58)}\). There are minimal studies measuring fructose intake and acute uric acid production, with the majority considering these two variables being observational cohort studies. In published intervention studies there is a crucial limiting factor to the current research in that the amounts of fructose that have been tested in the studies are much higher than that consumed by the majority of the population. As the role that uric acid plays in human physiology is unclear, there is a need to investigate the acute dose-response effect of serum uric acid concentrations on consumption of common serving sizes of sugar-sweetened beverages.

Given the current evidence of the role of fructose in uric acid production, and its high relevance to New Zealand, this study was conducted to assess fructose intake and uric acid production in healthy young adults.
3 Objective Statement

There is very limited research examining the effect of commercially available sugar-sweetened beverages on acute plasma uric acid concentrations. The aim of this study was to determine if consuming common single-serve sizes of a commercially available sugar-sweetened beverage had any effect on acute plasma uric acid levels in a healthy New Zealand population.

The study objectives are:

- To establish the fructose content in a popular single serve sugar-sweetened beverages.
- To determine if the body’s plasma uric acid response is different over a 60-minute period between the commercially available sugar-sweetened beverage and the monosaccharide fructose and glucose beverages.
- To determine if body’s plasma uric acid response is different over a 60-minute period between two different volumes of a sugar-sweetened beverage.
Figure 4.1 Modified CONSORT crossover randomised control trial study design and participant flow diagram.
4 Methodology

Data collection took place in the Human Nutrition Undergraduate Laboratories at the University of Otago between February 27th and March 20th, 2015.

The University of Otago Human Ethics Committee approved this study. All information on the study was included in the ethics proposal (Appendix A), including the study requirements, study information sheet and consent form (Appendix B, C). Māori consultation was sought and gained by submitting a consultation form to Ngāi Tahu (Appendix D, E). Registration of this trial was done via the Australian New Zealand Clinical Trials Registry (trial ID: ACTRN12614000322639).

4.1 Participants

4.1.1 Recruitment

Participants for this study were a convenience sample of University of Otago undergraduate and postgraduate human nutrition students. Participants provided informed consent before commencing the study.

4.1.2 Exclusion Criteria

Participants were excluded from the study if they were pregnant or had self-reported food allergies, or were diagnosed with diabetes mellitus, cardiovascular disease, cancer or diseases of the digestive system.

4.2 Randomisation and Allocation

Participants were randomised to either the intervention or control group and to the order in which they received their allocated beverages. Block randomisation was used for males to control for a potential source of variability between sexes, ensuring equal sex distribution.
across the groups. Randomisation was undertaken using Stata Statistical Analysis Software (version 13.1, Stata Corporation 2008). Dr. Jill Haszard, a biostatistician in the Human Nutrition department at the University of Otago, completed the randomisation on consultation with the study team. Twenty-one participants were randomised to the intervention group, where they consumed a Sprite beverage, in a crossover design. Twenty-one participants were randomised to the control group, where they consumed a fructose beverage and a glucose beverage, in a crossover design.

4.3 Study Design

As illustrated in Figure 4.1, this study is a randomised, controlled crossover trial in which plasma uric acid concentrations were measured at baseline and up to one hour following the consumption of sugary beverages. Participants were randomised to either the control or intervention group.

The intervention group consumed a 355 ml Sprite can on one testing day and a 600ml Sprite bottle on the other in random order. The control group consumed a 600ml fructose control on one testing day and a 600 ml glucose control on the other in random order.

Within this study, another Master of Dietetics (MDiet) student examined what affect the form of fruit, whole or juiced, has on plasma uric acid concentration. A Master of Science student also examined fructose intake and acute blood pressure response. These results are not reported in this thesis.

4.3.1 Sprite Beverage

The sugary beverage chosen for the study was Sprite (Coca-Cola Amatil Ltd, Auckland New Zealand). It was selected over other beverages, as it was milder in taste, more appealing and easier to consume in the 10 minute allocated consumption period. This study was designed to test whether sugary beverages consumed in typical amounts affected uric acid concentrations.
Two ‘single-serve’ volumes available in a local supermarket (355ml cans and a 600 ml plastic bottles) were used.

A can of Sprite was sent to Cawthron Institute in Nelson, New Zealand for the determination of fructose content using an in-house method based on published methodology (Appendix F). The laboratory provided a sugar profile analysis (Appendix G). The fructose content found was then matched to the sugar concentration of the fructose and glucose control beverages.

The total fructose content of Sprite was 4.45 g/100 ml; this comprised 0.8 g/100 ml of free fructose (monosaccharide) and 7.3 g/100 ml of sucrose, of which half (3.65 g/100 ml) was fructose, corresponding to a total 26.7 g of fructose per 600 mL bottle.

Further Sprite samples (a bottle and a can) that were representative of what the study participants consumed were sent to Cawthron Laboratories to check variability between samples. The results for fructose concentrations were within 1.0 g/600 ml of the original sample that was tested, and between the bottle and can. The fructose concentrations were 27.6 g/600 ml and 27.3 g/600 ml for the can and bottle respectively (Appendix H).

**4.3.2 Fructose and Glucose Controls**

The control test foods were comprised of a fructose beverage containing 26.7 g of fructose monosaccharide as a positive control, and a glucose beverage containing 26.7 g glucose monosaccharide as a negative control, as no plasma uric acid response was expected. The study coordinator (ELC) performed the weighing of the fructose and glucose powders and a lab assistant at the University of Otago added the carbonated water as the participants arrived. Hot water was used to dissolve the glucose to allow for easier mixing. Fructose or glucose equaling 26.7±0.2 g was added to carbonated water to make up a 600 ml beverage.
4.4 Study Procedure

4.4.1 Data Collection

Participants attended two testing days, both on Fridays, separated by either one or two weeks. The washout period was either one or two weeks due to participant availability and not because a washout period of this length was required. As it was the acute response being examined, one day between the tests would have been sufficient to ensure no carryover effect.

4.4.2 Demographic Screening Questionnaire

All participants (n=42) completed a baseline demographic screening questionnaire at the beginning of each testing day (Appendix I, J). The baseline were collected included unique ID, date of birth, contact details, self-identified ethnicity, current sweetened beverage and fruit juice intake, medication and supplement use, relevant medical history and smoking status. The second testing day questionnaire (Appendix J) asked similar information, with further detail on potential changes in dietary or physical activity habits, or in medication and supplement use since the previous testing day.

4.4.3 Testing Day Procedure

The day prior to the testing day, participants were asked to abstain from consuming alcohol or participating in vigorous exercise, to fast (refrain from consuming any food or liquid other than water) from 10pm the night before and to have an evening meal containing carbohydrate. A communication strategy was implemented to ensure all participants understood and were informed of the study requirements to increase compliance (Appendix K).

Upon arriving on the testing day, the participants signed a consent form to participate in the study (Appendix C). Baseline blood pressure was taken, followed by baseline blood samples. Once the first blood sample was collected, start times were recorded on a whiteboard and
participants were asked to consume their allocated beverage within a 10-minute period. Blood samples were collected at 30 and 60 minutes after the start of the ingestion period. Blood pressure was measured again after the final blood collection. Blood collection took place at 30 and 60 minutes as this was identified in the literature search as the most likely time to capture the biggest rise in acute plasma uric acid (8, 51). Height and weight measurements were collected and used to calculate body mass index (BMI = weight (kg) divided by height squared (m²)). Weight measurements were taken using calibrated Seca alpha scales (model 770). Height measurements were taken using a calibrated Holtain Limited stadiometer.

4.4.4 Sample Collection

Capillary blood samples were collected from all participants following an overnight fast. A 5 µL sample of blood was collected for glucose testing and a larger 500 µL sample of blood was collected for uric acid analysis. A blood collection protocol (Appendix L) was used and trained phlebotomists collected all blood samples.

Heat was applied to the hands to aid peripheral blood flow, and fingers were sanitised with alcohol wipes and then pricked using a disposable BD microtainer® contact-activated 2.0x1.5mm blue lancet. The first drop of blood was not used to avoid contamination or dilution from the alcohol wipe. The 500 µl blood sample was obtained first and stored in 1mL ‘purple top’ BD microtainer® tubes with K₂EDTA as an anti-coagulant.

A further drop of blood was collected for glucose analysis and was drawn out with a glucose 201 micro cuvette. A HemoCue glucose 201+ was used to determine glucose concentrations. The HemoCue self-calibrated through a control cuvette was used weekly to check the self-calibration was accurate. The results of the blood glucose test were self-recorded by the participants on the glucose recording sheet provided (Appendix I, J).
Blood samples were collected and refrigerated for up to 7 hours. They were centrifuged for 5 minutes at 2000 G to separate the plasma, which was then pipetted into labelled microcentrifuge tubes and stored at -80 degrees Celsius for less than one month.

4.4.5 Plasma Analysis

Plasma uric acid was determined using the Cobas c 311 auto-analyser (manufactured by Roche), undertaken in the Diabetes and Lipids Laboratory at the University of Otago, New Zealand. Once samples were defrosted, uric acid determination occurred within two hours. The Cobas c 311 used a calorimetric enzymatic test to determine the uric acid concentration (Appendix M). Quality control was ensured by using manufacturer controls; Precinorm U (PNU), Precipath U (PPU), control for all substrates (CFAS) and a pooled plasma sample of known uric acid concentration. A two-point calibration and within-instrument quality control were conducted. Calibration occurred before sample analysis, and again when the reagents were replaced. PPU, PNU and pooled plasma were run at the beginning of each day and pooled plasma was run with each 20-sample batch. Coefficients of variation were 2.31%, 2.71% and 2.55% for PNU, PPU and pooled plasma respectively.

The Cobas c 311 required a minimum of 120 µL of blood plasma to run the uric acid assay. Within our samples, 13% did not meet this level and required dilution. An 11-point dilution curve was plotted which determined a linear relationship, ensuring that uric acid concentration could be accurately determined when a sample was diluted (Appendix N). Sample dilutions ranged from 80:40 µL to 30:90 µL (plasma: distilled water).

Uric acid concentrations >410 μmol/L were considered high and were retested to ensure there was not a misreading (0.4% samples retested). If a high reading occurred, samples from the same study ID were monitored to see if readings were consistent and if so were not retested. If the secondary value was consistent, then the initial value was used. No triplicates occurred.
4.5 Statistics

A P-value of <0.05 was considered as statistically significant. However, both effect sizes and p-values were considered in the interpretation of results. Microsoft Excel (version 14.4.8) was used for calculating the incremental area under the curve (iAUC) and the analysis of continuous variables, including means and standard deviations and categorical data, including percentages. Ethnicities were prioritised using Ministry of Health ethnicity data protocols (59). Uric acid data were analysed using Stata Statistical Analysis Software (version 13.1, Stata Corporation 2008).

4.5.1 Sample Size Estimation

The size of the study population was based on the number of participants needed to provide statistical power to detect a difference in the primary outcome variable, plasma uric acid, measured in µmol/L between groups. A clinically significant difference was determined as 50 µmol/L between the two interventions. The study had 80% power to detect this difference in plasma uric acid between the two groups if the size of the groups was n=20. This sample size allowed for identifying differences in the uric acid response over time to different levels of fructose intake within groups.

4.5.2 Uric Acid Statistical Analysis

There were four values in the data set that were considered outliers. All four values were deemed to be physiologically unlikely when compared against the other values for that participant. One outlier was a baseline value and was replaced with the other baseline value collected on the second testing day. The other three outlying values were set as missing data. The primary outcomes of this study were the change in plasma uric acid concentration at 30 and 60 minutes after baseline and iAUC. To analyse the mean change in plasma uric acid in response to treatment, a linear mixed effects model was used with time as a fixed effect and
participant ID as a random effect. Uric acid trajectories over time were plotted for each individual and then the mean at each time point was plotted and superimposed on the individual trajectories. Mean uric acid was analysed between treatment groups using analysis of covariance and p-values.

The trapezoidal method was used to calculate iAUC. The iAUC analysis comparing between doses and sugars within groups was done by using a mixed effects model with dose as the fixed effect, and participant ID as the random effect. The iAUC analysis comparing between groups was done by using a multivariate linear regression with iAUC as the response variable and group as the predictor variable.

For both mean difference and iAUC, the treatment effect was adjusted for; order of intervention (high dose/low dose, glucose/fructose), time of the morning the testing day was attended, smoking history, sex, family history of gout and whether or not they had consumed alcohol the night before. Residuals for the statistical models were plotted and visually assessed for homogeneity of variance for the statistical models.
5 Results

Data from 41 participants were included in the data analysis. The randomisation, screening and exclusion of participants can be seen in Figure 4.1. Participants who took part in only one testing day were included in the analysis on an intention to treat basis.

5.1 Group Demographic Comparison

The baseline characteristics of the Sprite and control groups are presented in Table 5.1. Male BMI was higher in the Sprite group, compared to the control group, this was due to one BMI value being considerably larger, and when removed the difference in BMI average between the two groups was reduced. The groups were predominantly comprised of females, with block randomisation being used to ensure the males were spread evenly across the groups.

5.2 Uric Acid

5.2.1 Evaluation of Plasma Uric Acid Concentrations

The mean baseline plasma uric acid level for the study cohort was 267 μmol/L, which ranged from 107 to 404 μmol/L. The formation of monosodium urate crystals starts to occur when serum uric acid levels reach 408 μmol/L (60). No participants exceeded this level during their baseline measurements. However, after consuming the beverages, there were three participants who had plasma uric acid concentrations rise above 408 μmol/L. These participants had consumed the fructose control and the 355 ml and 600 ml beverage.
Table 5.1 Baseline participant characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sprite (n=21)</th>
<th>Control (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (23.8%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (76.2%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td><strong>Ethnicity (prioritised)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European</td>
<td>16 (76.2%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Māori</td>
<td>1 (4.8%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (14.2%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (4.8%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22.6 (1.9)</td>
<td>22.2 (2.3)</td>
</tr>
<tr>
<td>Female</td>
<td>21.9 (4.2)</td>
<td>20.4 (1.2)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>180.5 (9.1)</td>
<td>177.3 (8.6)</td>
</tr>
<tr>
<td>Female</td>
<td>167.0 (5.1)</td>
<td>169.2 (6.6)</td>
</tr>
<tr>
<td><strong>Body mass (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83.1 (9.4)</td>
<td>70.8 (10.7)</td>
</tr>
<tr>
<td>Female</td>
<td>61.2 (6.3)</td>
<td>64.8 (5.2)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>25.6 (2.8)</td>
<td>22.5 (2.1)</td>
</tr>
<tr>
<td>Female</td>
<td>22.2 (1.9)</td>
<td>22.7 (2.2)</td>
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<tr>
<td><strong>Baseline blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male Systolic</td>
<td>121.5 (12.4)</td>
<td>122.7 (9.3)</td>
</tr>
<tr>
<td>Male Diastolic</td>
<td>61.7 (5.6)</td>
<td>65.3 (3.8)</td>
</tr>
<tr>
<td>Female Systolic</td>
<td>106.4 (8.1)</td>
<td>105.5 (7.4)</td>
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<tr>
<td>Female Diastolic</td>
<td>61.5 (5.8)</td>
<td>59.8 (5.3)</td>
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<tr>
<td><strong>Baseline blood glucose (mmol/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.1 (0.3)</td>
<td>4.8 (0.4)</td>
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<tr>
<td>Female</td>
<td>5.0 (0.4)</td>
<td>5.1 (0.6)</td>
</tr>
<tr>
<td><strong>Baseline serum uric acid (μmol/L)</strong></td>
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</tr>
<tr>
<td>Male</td>
<td>324.4 (76.9)</td>
<td>309.7 (31.7)</td>
</tr>
<tr>
<td>Female</td>
<td>240.7 (65.0)</td>
<td>254.6 (46.5)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoked</td>
<td>17 (81%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>Previous smoker</td>
<td>2 (9.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>2 (9.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Family history of gout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1 (4.8%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Female</td>
<td>3 (14.3%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

* Results presented as n (%)
† Results presented as mean (SD)
‡ Difference between groups is statistically significant P=<0.05
5.2.2 Adjusted Mean Difference Between Volumes, Sugars and Time

Table 5.2 Adjusted mean differences between baseline, 30 and 60 minutes*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Difference (μmol/L)</th>
<th>95% CI Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>-5.00</td>
<td>(-10.93, 0.93)</td>
<td>0.099</td>
</tr>
<tr>
<td>60 minutes</td>
<td>-8.37</td>
<td>(-15.30, -1.45)</td>
<td>0.018</td>
</tr>
<tr>
<td>Fructose control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>25.51</td>
<td>(10.95, 40.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>60 minutes</td>
<td>26.34</td>
<td>(8.56, 44.12)</td>
<td>0.004</td>
</tr>
<tr>
<td>Sprite 355ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>6.64</td>
<td>(1.56, 11.73)</td>
<td>0.010</td>
</tr>
<tr>
<td>60 minutes</td>
<td>4.38</td>
<td>(0.85, 7.91)</td>
<td>0.015</td>
</tr>
<tr>
<td>Sprite 600ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>7.70</td>
<td>(0.700, 14.70)</td>
<td>0.031</td>
</tr>
<tr>
<td>60 minutes</td>
<td>8.65</td>
<td>(1.30, 15.99)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

* Adjusted for the order of intervention, test time, alcohol consumption, smoking, sex and family history of gout.

Unadjusted and adjusted results were determined, with little difference being seen between the two sets of results. The adjusted model was more statistically robust, with greater precision and better model fit. Consequently, the adjusted results are presented. The results for the mean difference in plasma uric acid at 30 minutes and 60 minutes for each test beverage are presented in Table 5.2. The glucose and fructose control had opposite, but significant effects on plasma uric acid concentrations. The glucose beverage significantly decreased plasma uric acid concentration after 60 minutes (P=0.018) and the fructose beverage significantly increased plasma uric acid concentration after 30 minutes (P=0.001) and 60 minutes (P=0.004). The mean trends showing the negative and positive association for the control group are presented in Figure 5.1. There was a significant difference between the fructose and glucose beverages at both 30 and 60 minutes (P<=0.001) (Figure 5.1).
The Sprite beverage at both the higher and the lower volume significantly increased plasma uric acid concentration (Table 5.2). The 355 ml Sprite significantly increased plasma uric acid concentration at both 30 and 60 minutes post ingestion, however after 30 minutes it had started to decrease. The 600 ml Sprite saw a continual rise at both 30 and 60 minutes after ingestion. Across all the beverages and amounts, the fructose control had the most significant effect on plasma uric acid concentration at both 30 and 60 minutes post ingestion.

There was no difference in acute uric acid response found between the 600 ml and 355ml Sprite at either 30 or 60 minutes (P=0.745, 0.116) (Figure 5.2).

![Graph of individual plasma uric acid trajectories and mean plasma uric acid for the control group.]

**Figure 5.1** Individual plasma uric acid trajectories and mean plasma uric acid for the control group.
The data presented in Figure 5.1 and Figure 5.2 show the between-person variation in plasma acid concentrations are large and range from 169-354 μmol/L and 107-404 μmol/L for the control and Sprite groups respectively. The within-person variation is small with the average increases ranging from 4.38-26.34 μmol/L as presented in Table 5.2.

**Figure 5.2** Individual plasma uric acid trajectories and mean plasma uric acid for the Sprite group.
5.2.3 Incremental Area Under the Curve

Table 5.3 Adjusted iAUC for plasma uric acid difference in groups between intervention beverages and sugars*.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>iAUC (mmol/l.min)</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within Groups†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control – Glucose and Fructose</td>
<td>1349.65</td>
<td>(750.03, 1949.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sprite – 355ml and 600ml</td>
<td>103.53</td>
<td>(-196.77, 403.83)</td>
<td>0.499</td>
</tr>
<tr>
<td><strong>Between Groups‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprite 600ml and Glucose</td>
<td>645.41</td>
<td>(133.60, 1157.23)</td>
<td>0.015</td>
</tr>
<tr>
<td>Sprite 600ml and Fructose</td>
<td>-727.79</td>
<td>(-1643.25, 187.66)</td>
<td>0.114</td>
</tr>
</tbody>
</table>

* Adjusted for the order of intervention, test time, alcohol consumption, smoking, sex and family history of gout
† Mixed-effects model was used, and adjusted values are presented
‡ Multivariate linear regression was used, and adjusted values are presented

The fructose and glucose sugars revealed different responses in plasma uric acid. As shown in Table 5.3, iAUC was significantly different between the two sugars (P=<0.001) though no difference in iAUC was seen between the Sprite beverages (P=0.499).

The iAUC between the Sprite 600 ml and the two control beverages varied (Table 5.3). Comparing the fructose beverage to the 600 ml Sprite, there was no significant difference in iAUC between the two groups (P=0.114). However, when comparing the glucose beverage to the 600ml Sprite, a significant difference was observed (P=0.015). Looking within Sprite group there was no significant difference between the 355 ml and 600 ml amounts (P =0.499). However, there was a significant difference between the fructose and glucose control beverages (P=<0.001).
6 Discussion

The objective of this study was to determine the effect of two commonly consumed single-serve amounts of a sugar-sweetened beverage on acute plasma uric acid concentration in a sample of healthy young New Zealand adults.

Plasma uric acid concentration increased following consumption of the fructose control and the Sprite beverages, whereas it decreased after consumption of the glucose beverage. There was no apparent dose-response between the two Sprite volumes. However, our study may not have been able to detect a difference due to the large inter-individual variation in plasma uric acid concentrations. There was an indication that dose had an effect in that the increased uric acid concentrations were maintained for 60 minutes with the fructose control and the larger Sprite whereas it had started to fall after 30 minutes with the smaller volume. Our results clearly showed that the body’s response to fructose and uric acid production is fast.

These results are novel in that uric acid concentration is known to increase following the consumption of large amounts of fructose\(^{(25)}\), however, this is the first study to report plasma uric acid changes following the consumption of commercially available beverages consumed in single-serving sizes.

6.1 The Fructose Argument

Some people are highly opposed to added fructose consumption, suggesting that it is a major contributor to chronic disease\(^{(15,61)}\). Others are more circumspect, suggesting that a problem occurs only when excessive amounts of fructose are consumed from foods containing fructose in the commonly consumed forms of sucrose or HFCS\(^{(14,37)}\).

Focusing on fructose and uric acid concentrations, several cohort studies have found that higher fructose intakes are associated with an increased risk of hyperuricemia and gout\(^{(3,7,24)}\). Whereas a meta-analysis of observational studies investigating associations between fructose,
consumed within the recommended energy intake, and serum uric acid concentrations have concluded that fructose does not chronically raise serum uric acid \((16)\).

Our study, which focused on the acute setting, found that fructose significantly raised plasma uric acid. This result suggests that reasonable amounts of fructose, found in single serve sugar-sweetened beverages, acutely cause a small and transient rise in plasma uric acid. Our study tested a sucrose-sweetened beverage and these results may differ when using a HFCS-sweetened beverage, which may be of particular concern in North America, where HFCS is more commonly used.

6.2 Fructose Dose

Our study focused on the use of commonly bought 355 ml and 600 ml sugar sweetened beverages that are consumed on a regular basis and are widely available in New Zealand. The doses were vital to the design of our study. Previous literature \((21, 62)\) tested fructose amounts equaling as much as 25\% of average daily energy intake \((21)\) or 150g of sugar per day \((62)\), far exceeding the 10-12\% or 103-129g estimated to be the average intake in New Zealand \((13)\).

6.3 Serum Uric Acid Response in the Body

The fructose and glucose controls produced different serum uric acid responses. Differences in the metabolism of these two sugars can account for the observed responses. When metabolised, fructose is rapidly phosphorylated, ultimately activating the catabolic pathway resulting in uric acid production \((11, 33)\).

The fructose control increased plasma uric acid concentrations, which has been found in other literature \((26)\). It would be unusual for people to consume fructose alone and not as a component of food and, therefore, the results should be interpreted with caution \((37)\).

Our results found the glucose control beverage induced a decrease in plasma uric acid concentration. Decreases have been observed in other literature where glucose was administered at 25\% of daily energy and observed serum uric acid decreasing by 23 \(\mu\)mol/L.
In contrast, an earlier study observed that serum uric acid concentrations did not change when glucose was administered at 1 g/kg body mass. There is no current metabolic pathway suggesting that glucose decreases serum uric acid. However, based on the observed decrease in our study it could be possible that when glucose and fructose are consumed simultaneously, the rise in uric acid induced by fructose is partially counteracted by the glucose, resulting in a smaller rise.

The decrease we observed in glucose could plausibly be explained by diurnal variation in serum uric acid. Researchers have found that serum uric acid increases during sleep. Another study has shown that serum uric acid concentrations are raised in the morning and tend to trend downwards as the day continues. Our testing was done in the morning and thus it is possible that at baseline measurement our participants had raised uric acid concentrations, which were naturally declining over the time course of the experiment. The implications of serum uric acid diurnal variation could mean that had we tested the Sprite beverages at a time later in the day, the rise that was seen may have been different.

After consuming the 600 ml Sprite, the body’s plasma uric acid response was larger compared to the glucose control. This result makes physiological sense as only the Sprite beverage contains fructose.

The body’s plasma uric acid response was no different between the fructose control (100% fructose) and 600 ml Sprite (50% fructose). As one beverage was a fructose monomer and the other was fructose bound with glucose in the form of sucrose, this finding suggests the hydrolysis of sucrose plays no role in slowing down the fructose metabolism. It also indicates that fructose, whether free or in sucrose, is absorbed and metabolised in a similar manner.

When considering iAUC, the fructose control and the 600 ml Sprite produced a noticeable though statistically insignificant difference. The trend we observed in our iAUC data between the 600 ml Sprite and fructose control indicates the fructose beverage may, in fact, generate a higher plasma uric acid response in the initial hour post consumption. This study’s sample
size was determined on the difference in uric acid at each time point and may have been underpowered to detect a difference between iAUC.

There was no dose-response between the two Sprite beverage servings, despite being 10.9g different in sugar content. No other studies have been published using different single serving sizes of the same commercial beverage; therefore this finding is novel. Differences in the way each volume was consumed may have masked a significant dose-response effect. The 355 ml can was a more manageable volume for most of the participants and was generally consumed within the 10-minute ingestion period. The 600 ml bottle often took longer than 10 minutes and participants tended to sip this, rather than drink it quickly.

6.4 Clinical Implications

These results may prompt some debate as to whether a small and transient rise in plasma uric acid, such as what was found in this study, has any clinical relevance. Some might argue that uric acid is harmful and can increase the risk of developing gout\(^\text{(10)}\). Whereas others might take the opposite view, that uric acid is a useful antioxidant\(^\text{(67)}\). Uric acid has been identified as a powerful antioxidant in the epithelial cells in the body\(^\text{(68)}\). Throughout evolution, uricase, an enzyme involved in the metabolism of uric acid, has mutated at multiple points in time rendering humans and some great apes unable to metabolise uric acid\(^\text{(69)}\). These mutations are suggestive of an evolutionary advantage in being able to maintain uric acid at concentrations higher than what would otherwise have been the case had uricase been functioning\(^\text{(69)}\).

Gout is a disease caused by raised uric acid concentrations\(^\text{(10)}\). Some members of the scientific community believe that sugar-sweetened beverage intake is associated with increased risk of gout and can act as a trigger for gout symptoms\(^\text{(7, 10, 24)}\). However, this is unlikely in our study population, which was young and had relatively low plasma uric acid concentrations. For a more metabolically at risk population, who consume higher amounts of sugar-sweetened beverages, our findings may pose more of a problem.
Approximately three-quarters of serum uric acid is excreted by the kidneys, with the remainder being handled by the gastrointestinal tract. Gout is a disease that is “principally caused by inefficient renal urate excretion.” Those who suffer from gout often have a reduced ability to excrete uric acid through renal pathways. Diuretics play a large role with increased risk of gout with studies finding a strong association between the use of various diuretics and the increased incidence of gout. Diuretics are thought to decrease uric acid excretion and increase uric acid reabsorption.

Aside from physiological mechanisms, dietary factors are also believed to have a role in influencing serum uric acid. High purine foods, including protein rich foods are believed to have the largest effects on serum uric acid, with shellfish, poultry, red meat, fish, alcohol and fructose being associated with increased incidence of gout. Dairy, vitamin C and coffee are all believed to have a uricosuric effect, thereby increasing renal excretion.

Our results showed an average serum uric acid increase in the 600ml Sprite of 8.65 µmol/L. Within individuals, the largest rise was 110µmol/L. However, most participants increased by much less (<40 µmol/L). Previous literature has presented varied serum uric acid responses, possibly due to the variety of fructose dose and length of follow-up. Longer-term studies using fructose at 10% of daily energy requirements over a 16-day period determined a similar increase in uric acid to what our results suggest. Differences as large as 72.49 µmol/L have been seen in a 10-week study using fructose at 25% of daily energy intake. An acute study tested sucrose-sweetened beverages with 75 g of sucrose (37.5g of fructose) in a 300ml beverage saw an increase of 20 µmol/L after 60 minutes. I hypothesise the duration of hyperuricemia may be related to the dose of fructose that is used, as the studies using higher doses with longer follow-up periods seem to identify larger rises in serum uric acid.
6.5 Strengths and Limitations

Strengths of this study are found when you consider the gaps in the previous research. A fundamental strength of this study was that a commercially available beverage was used in servings sizes that are commonly consumed by the population. Using this type of beverage ensures translatability in our results. Additionally, the 355 ml Sprite contained 15.8 g of fructose, and acute uric acid responses to this low amount have not been previously reported.

Using a fructose and glucose control was a strength of our study design as we could determine it was the fructose in the Sprite beverage increasing plasma uric acid and not the glucose or other beverage additives. The increase observed in the fructose control is consistent with the hepatic metabolism of fructose and strengthened the association between fructose ingestion from the Sprite and the rise in plasma uric acid.

Another strength was having the Sprite beverage analysed to determine the fructose content. The amount of fructose and glucose in the control beverages was then matched to the amount in a 600 ml bottle of Sprite. After data collection, we had further samples tested to ensure the fructose content did not vary too much between a bottle and can and between batches. We observed minimal differences between the three samples we had tested (<1 g/600 ml).

Methodological strengths included; a crossover study design, participants were asked to fast to minimise confounding, all uric acid analysis was completed by the study coordinator (ELC), and where possible each person had their blood collected by the same phlebotomists at each testing day. Participants were all healthy, young adults, which suggests the rise observed is a normal physiological response, whereas in an older population, it could have been due to impaired renal or metabolic function.

A limitation for this study was that the study population was a convenience sample chosen for their accessibility. Our study was conducted in healthy, young adults, predominantly female (76%), at low-risk of gout. Hence, our data may have limited generalisability to an older,
more metabolically at risk population, particularly if uric acid production differs by age or health. However the concept that fructose, when consumed in realistic amount, raises uric acid production is probably transferable across the life cycle. Another limitation was that our small sample size and large variability between participants might have lead to a type II error. Our results also make it unclear whether there is a dose-response with fructose and plasma uric acid production or if there is a plateau effect.

Future acute studies measuring fructose intake and uric acid production would benefit from a more generalisable study population, with a larger sample size, even sex distribution and a widespread age range. A further study in New Zealand could consider testing fructose in line with current average intakes of 8-10% of added sugars\(^{(13)}\), or in line with the sugar guidelines released by the World Health Organisation earlier this year, which aim to reduce added sugar intake to 5% of your daily energy\(^{(12)}\).

6.6 Conclusion

Although differences in the metabolic fate of glucose and fructose have been well characterised, this is the first demonstration reflecting this difference using commonly consumed serves of a typical commercially manufactured sugary beverage. Our results determined that after consuming a fructose control; 355 ml and 600 ml Sprite beverage resulted in a statistically significant rise in plasma uric acid in a healthy population of young adults. Glucose had no effect on raising plasma uric acid and our results showed a decrease over 60 minutes. The body’s plasma uric acid response did not significantly differ between the 600 ml Sprite and the fructose control though it is possible that this lack of significance is a type II error.

As our results showed an increase after consuming both single serving sizes of a sugar-sweetened beverage, it is recommended that those at risk of or suffering from gout monitor and if necessary reduce their sugary beverage intake.
7 Application to Dietetic Practice

For individuals suffering from hyperuricemia or gout, the gold standard treatment entails pharmaceutical interventions, such as allopurinol, along with the dietary restriction of foods, which are thought to influence symptoms\(^{(75)}\). Restricting purine-rich foods i.e. red meat and seafood, the non-consumption of alcohol and adequate hydration are dietary factors that have robust epidemiological support\(^{(7,10,44,76,77)}\). These guidelines have been the mainstay of dietary gout management for many decades\(^{(10)}\). Novel research has emerged over the years associating sugar-sweetened beverages and fructose consumption to an increase in the risk of developing gout and symptom severity\(^{(10)}\). The efficacy of prescribing lifestyle interventions to treat gout has been questioned due to the lack of supportive studies, though the consensus is that healthy lifestyle advice is appropriate\(^{(75)}\).

From the present study, serum uric acid rose acutely after consuming the 355 ml or 600 ml serving of Sprite, single serving sizes. Restricting oral intake of sugar-sweetened beverages and fructose for gout management should be encouraged at an individual basis. Gout symptom triggers vary between individuals, therefore, advice to reduce sugary beverage and fructose intake may provide substantial improvement for some. Reducing the intake of sugary beverages and fructose may not only reduce gout symptoms, but could also remove a source of excess energy from the patient’s diet. Obesity is correlated with gout\(^{(78)}\). Reducing excess energy intake could have a positive effect on weight management and severity of gout symptoms.

Restricting your diet can often be a very difficult challenge, particularly if you have to limit foods that you commonly consume. Diet beverages could be suggested as an alternative to normal sugary beverage consumption. Artificial sweeteners have no effect on serum uric acid levels and, therefore, can very likely be consumed without inducing symptoms\(^{(7)}\).
From a public health perspective, restricting sugary beverages in gout management would be a beneficial campaign. Aside from fruit, most foods that contain added fructose have a limited nutritional benefit and result in excess energy consumption leading to obesity and its comorbidities.

It is important for the dietetic profession to emphasise the lack of nutritional value that sugary beverages provide beyond energy and to highlight healthier alternatives such as water and low-fat milk. This research has shown that serum uric acid increases in response to sugar-sweetened beverage consumption. Reducing sugar-sweetened beverage consumption could aid in reducing a magnitude of adverse health outcomes, such as obesity, diabetes, gout and related comorbidities.
References


9 Appendices

Appendix A: Ethics Proposal
Appendix B: Study information sheet
Appendix C: Study consent form
Appendix D: Māori consultation submission form
Appendix E: Māori consultation response
Appendix F: Cawthron Laboratories in-house method
Appendix G: Cawthron Laboratories sugar profile analysis (pretesting)
Appendix H: Cawthron Laboratories sugar profile analysis (post testing)
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Appendix J: Baseline screening questionnaire (second testing day)
Appendix K: Study communication strategy
Appendix L: Blood collection protocol
Appendix M: Cobas c 311 uric acid analysis procedure
Appendix N: Dilution curve
Appendix A: Ethics proposal

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 (Dr)
   Venn        Bernard

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
   Student Researchers
   Names: Sara White MDiet
   Level of Study (PhD, Masters, Hons):
   External Researchers
   Institute/Company:
   Names: Emma Carran MDiet
   Andrew Reynolds PhD

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
In the event of an abnormal result, a repeat finger-prick 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 milliliters. 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.

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.
Appendix B: Study information sheet

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 and five minutes after, a 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 finger pricks 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 lockable 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.

**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.
Appendix C: Study consent form

HUNT311 clinical nutritional laboratory; a repeated teaching activity

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. Finger prick 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.
Appendix D: Māori consultation submission form

Principal Investigator 1
Name: Dr Bernard Venn
Department: Department of Human Nutrition
Campus: DUNEDIN
Email: bernard.venn@otago.ac.nz

Principal Investigator 2
Name: Aspro Tony Merriman
Department: Department of Biochemistry
Campus: DUNEDIN
Email: tony.merriman@otago.ac.nz

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 Maori is one of the highest in the world (Te Karu et al. Maori experiences and perceptions of gout and its treatment: a kaupapa Maori qualitative study. J Prim Health Care 20135:214-222). 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 Maori 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
Appendix E: Māori consultation response

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE
TE KOMITI RAKAHAU KI KĀI TAHU

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 Ōnīkou Incorporated
Kāti Huirapa Rūnaka ki Puketeraki
Te Rūnanga o Moeraki
Ngāi Tahu Research Consultation Committee
Te Komiti Rakahau ki Kai Tahu

Nīhaku noa, nā

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

The Ngāi Tahu Research Consultation Committee has membership from:
Te Rūnanga o Ōtākou Incorporated
Kāti Huirapa Rūnaka ki Paketareaki
Te Rūnanga o Moeraki
Appendix F: Cawthron Laboratories in-house method

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 ultra-performance 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. MacRoeed; Academic Press, 1982.

Appendix G: Cawthron Laboratories sugar profile analysis (pretesting)
# Appendix H: Cawthron Laboratories sugar profile analysis (post testing)

## Certificate of Analysis: Final

**Project Number:** T50137  
**University of Otago**  
**PO Box 56**  
**DUNEDIN**  
**Attention:** Emma Carran

**Customer Order No:** 9860 FM  
**Email Recipients:** Emma Carran

**Sample Details**

**Laboratory ID:** T50137-1  
**Description:** Sprite 355 ml Can  
**Sample Type:** Product  
**Date Sampled:** 26/03/2015 13:30  
**Date Received:** 28/03/2015 09:00

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Result</th>
<th>Units</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>0.6</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.7</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Maltose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Mannose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.0</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Xylose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
</tbody>
</table>

**Sample Details**

**Laboratory ID:** T50137-2  
**Description:** Sprite 600ml Bottle  
**Sample Type:** Product  
**Date Sampled:** 26/03/2015 13:30  
**Date Received:** 28/03/2015 09:00

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Result</th>
<th>Units</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>0.5</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.6</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Maltose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Mannose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
<tr>
<td>Xylose</td>
<td>&lt;0.1</td>
<td>g/100g</td>
<td>In House Method</td>
</tr>
</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:** 9/4/15

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* Indicates an analysis that is not IANZ accredited

**Report Number:** 573263  
**Project Number:** T50137

---

T (+64) 03 548 2839 | F (+64) 03 546 9464 | 98 Halifax Street East, Nelson 7010. Private Bag 2, Nelson 7042, New Zealand | www.cawthron.org.nz

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Appendix I: Baseline screening questionnaire (testing day 1)

Information Collection Sheet – Week 1

<table>
<thead>
<tr>
<th>Student ID Number: ______________________</th>
<th>Male/ Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of Birth: __________</td>
<td>Age: __________</td>
</tr>
</tbody>
</table>

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

Have you eaten anything from 10pm last night? YES/NO
If yes, what did you eat?

Did you have a carbohydrate-based meal for dinner last night? YES/NO

Did you drink alcohol yesterday or this morning? YES/NO
If yes, how many standard drinks did you consume? E.g. ½ glass of wine, one can of beer, one single shot.

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 Maori
☐ 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 possibly pregnant? (if female) YES/NO

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

If you have been diagnosed with any disease of the digestive system then please list them:
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 or alcoholic RTD's? (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 J: Baseline screening questionnaire (testing day 2)

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 did you eat?

Did you have a carbohydrate-based meal for dinner last night? YES/NO

Did you drink alcohol yesterday or this morning? YES/NO
If no, how many standard drinks did you consume? E.g. ½ glass of wine, one can of beer, one single shot.

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.
Appendix K: Study communication strategy

Strategy to ensure information is relayed to participants:

- To be put on blackboard: Information sheet, lab timetable and ethical consent form. ASAP
- Once students are enrolled and streamed they will receive an email from Andrew containing information about: lab streams, information sheet, pre-lab instructions and contact details if they have a timetable clash.
- In lectures with Bernard Thursday each week: Data collection (day before testing day): Verbal reminder to attend and pre-lab instructions.
- At lab: Ethics consent form, information sheet and fill-in form for self-reported baseline details.

Email from Andrew prior:

Please check out the lab streams for this semester, remembering that a lot of you start labs this Friday. Should you have a clash with another paper please contact me immediately: andrew.reynolds@otago.ac.nz.

A few notes about your Carbohydrate labs being run over the next few weeks:

- These labs are fasted – consume no food from 10pm the night before (medications and water are ok)
- Eat a meal containing a decent amount of carbohydrate the night before (potato, pasta, rice or bread based)
- Don’t got overboard on the training in the 24 hours before the lab – do no more than what you consider an average level of physical activity
- Do not consume alcohol for at least for the 24 hours preceding each lab
- You have been randomised to receive one of three drinks or a fruit as part of this lab – we are going to see what this food does to your glucose, uric acid and blood pressure levels. Please contact me if you are diagnosed with fructose malabsorption or have a condition that makes fasting risky
- Wear something so you can roll up your sleeve to bare arm so we can take your blood pressure
- There will be a small quiz at both lab each worth 2.5% of your end result for 311
- During the lab we are going to take a 500-microliter sample from your finger three times! This will be fun, but may result in slight bruising of your fingertips. We’ll use heat bags and trained staff to minimise your discomfort, please contact me before the labs if this may be an issue for you. If you know that you are a slow capillary bleeder or suspect it might be the case, it is up to you to utilise heat bags as much as possible on your fingers in the 10 minutes before each blood draw.
Appendix L: Blood collection protocol

Student arrives at your station

1. Say hello and be friendly. People get nervous around blood.
2. Explain that you’ll be taking a sample and that you may need their help massaging their forearm to move blood towards their fingers.
3. Ensure you use one of the middle three fingers on either hand that has had a heat bag applied.
4. Wipe the finger with an alcohol wipe.
5. Prick with the lancet and use the square wipe quickly to clear away the initial blood (alcohol dilution of blood).
6. Collect sample into mini tube – go for at least 375ul, but ideally 500ul. When collecting the sample, don’t ‘milk’ the finger too hard as you may dilute the sample with interstitial fluid.
7. To collect the blood glucose sample use the micro cuvette by applying the very tip of it to a drop of blood. Wipe the outside of the micro cuvette with the square wipe and place in Hemocue.
8. Invert mini tube with lid on 7-9 times so that the blood mixes with the anticoagulant coating inside the tube.
9. Give participant cotton swab or band aid to clean up their finger.
10. Store blood sample in a chilly bin, with no ice.
Appendix M: Cobas c 311 uric acid analysis procedure

Order information
Uric Acid ver2
400 tests
Calibrator f.a.s. (12 x 3 mL)
Cat. No. 03183807 190
Calibrator f.a.s. (12 x 3 mL, for USA)
Cat. No. 0795950 190
Precinorm U plus (10 x 3 mL)
Cat. No. 12149435 122
Precinorm U plus (10 x 3 mL, for USA)
Cat. No. 12149438 160
Precipath U plus (10 x 3 mL)
Cat. No. 12149443 122
Precipath U plus (10 x 3 mL, for USA)
Cat. No. 12149443 160
Precipath U (20 x 5 mL)
Cat. No. 1017174 122
Precipath U (20 x 5 mL, for USA)
Cat. No. 1017177 122
Diluent NaCl 9 % (50 mL)
Cat. No. 04489357 180
System-ID 07 6615 1
Rochester Hitachi cobas c systems

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; zucchini)
≥ 83.5 μkat/L (25 °C); stabilizers
R2 Phosphate buffer: 0.1 mol/L, pH 7.8; potassium hexanoylamide (II): 0.3 mmol/L; 4-aminozenone ≥ 3 mmol/L; uricase (EC 1.1.1.17; horse) ≥ 83.4 μkat/L (25 °C); peroxidase (POD) (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
10 Shelf life at 2-6 °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 2-6 °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: L-heparin and K3-EDTA plasma
EDTA plasma values are approximately 7 % lower than serum values.

The samples types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Urine: Assay urinary uric acid as soon as possible. Do not refrigerate.
To prevent urate 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.
Application for urine

cobas c 311 test definition
Assay type 2 Point End
Reaction time / Assay points 10 / 23-27
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units mg/dL (µmol/L, mg/L)
Reagent pipetting Diluent (H₂O)
R1 72 µL 25 µL
R3 14 µL 20 µL
Sample volumes Sample Sample dilution
Normal 3 µL – –
Decreased 12 µL 15 µL 135 µL
Increased 6 µL – –

cobas c 501/502 test definition
Assay type 2 Point End
Reaction time / Assay points 10 / 34-42
Wavelength (sub/main) 700/546 nm
Reaction direction Increase
Units mg/dL (µmol/L, mg/L)
Reagent pipetting Diluent (H₂O)
R1 72 µL 25 µL
R3 14 µL 20 µL
Sample volumes Sample Sample dilution
Normal 3 µL 15 µL 150 µL
Decreased 3 µL 6 µL 160 µL
Increased 6 µL 15 µL 150 µL

Calibration
Calibrators S1: H₂O
S2: C.I.A.s.
Calibration mode Linear
Calibration frequency 2-point calibration
- after reagent lot change
- and as required following quality control procedures

Traceability: This method has been standardized against ID/MS. 07

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 should 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 5.5 = µmol/L
mg/dL x 10 = mg/L
**UA2**  
**Uric Acid ver.2**

**Limitations - Interference**

- **Criterion:** Recovery within 10% of initial value at uric acid concentrations of 7 mg/dL (417 μmol/L).
- **Serum/plasma**
  - Icterus: No significant interference up to an I index of 40 (approximate conjugated and unconjugated bilirubin concentration: 664 μmol/L (40 mg/dL)).
  - Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 μmol/L (1000 mg/dL)).
- **Lipemia (triglycerides):** 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 mmol/L < 3 mg/dL) does not interfere.
- Drugs: No interference was found at therapeutic concentrations using common drug panels. Exceptions: Calcium dobesilate, Levodopa and methyldopa can cause falsely low uric acid results.
- Uncase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

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

**Urine**

- Drugs: No interference was found at therapeutic concentrations using common drug panels.
- Exceptions: Calcium dobesilate, Levodopa and methyldopa can cause falsely low uric acid results.
- High homocysteic acid concentrations in urine samples lead to false results.

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

**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/DMS/Multiclean SSCS or the NaOH/DMS/SmpChnl + 2/SCCS Method Sheets. For further instructions refer to the operator manual.

**cobas c 501 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 (11.9-1467 μmol/L)

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

- **Urine:** 2.5-275 mg/dL (131-1639 μmol/L)

Determine samples having higher concentrations via the run function. Dilution of samples via the run function is 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)
  - Urine: 2.2 mg/dL (131 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).

**Expected values**

<table>
<thead>
<tr>
<th>Serum/plasma</th>
<th>Males: 3.4-7.0 mg/dL (202.3-416.5 μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females:</td>
<td>2.4-5.7 mg/dL (145.8-335.2 μmol/L)</td>
</tr>
</tbody>
</table>

**Urine** (reference range according to Kierg and Cowombo)

- **24-hour urine:**
  - Males: 200-1000 mg/day (1200-5900 μmol/day)
  - Females: 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:** 250-750 mg/24 hours
- **Low purine diet:**
  - Females: < 400 mg/24 hours
  - Males: < 480 mg/24 hours
- **High purine diet:** < 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:

<table>
<thead>
<tr>
<th>Serum/plasma</th>
<th>Repeatability* Mean mg/dL (μmol/L)</th>
<th>SD mg/dL (μmol/L)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precnorm U</td>
<td>4.54 (270)</td>
<td>0.04 (2)</td>
<td>0.9</td>
</tr>
<tr>
<td>Precpeth U</td>
<td>11.3 (660)</td>
<td>0.1 (6)</td>
<td>0.7</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>4.03 (240)</td>
<td>0.04 (2)</td>
<td>0.9</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>7.23 (430)</td>
<td>0.06 (4)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Mean mg/dL (μmol/L)</th>
<th>SD mg/dL (μmol/L)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precnorm U</td>
<td>4.47 (266)</td>
<td>0.07 (4)</td>
<td>1.6</td>
</tr>
<tr>
<td>Precpeth U</td>
<td>11.3 (660)</td>
<td>0.12 (6)</td>
<td>1.6</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>3.96 (236)</td>
<td>0.05 (3)</td>
<td>1.3</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>7.17 (427)</td>
<td>0.10 (6)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine</th>
<th>Repeatability* Mean mg/dL (μmol/L)</th>
<th>SD mg/dL (μmol/L)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>11.7 (696)</td>
<td>0.1 (6)</td>
<td>1.2</td>
</tr>
<tr>
<td>Control 2</td>
<td>21.7 (1291)</td>
<td>0.3 (16)</td>
<td>1.3</td>
</tr>
<tr>
<td>Urine 1</td>
<td>28.8 (1744)</td>
<td>0.6 (36)</td>
<td>2.1</td>
</tr>
<tr>
<td>Urine 2</td>
<td>32.5 (1934)</td>
<td>0.5 (30)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intermediate</th>
<th>Mean mg/dL (μmol/L)</th>
<th>SD mg/dL (μmol/L)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>11.4 (678)</td>
<td>0.2 (12)</td>
<td>1.9</td>
</tr>
<tr>
<td>Control 2</td>
<td>21.3 (1267)</td>
<td>0.3 (16)</td>
<td>1.6</td>
</tr>
<tr>
<td>Urine 3</td>
<td>29.3 (1743)</td>
<td>0.9 (64)</td>
<td>3.0</td>
</tr>
<tr>
<td>Urine 4</td>
<td>32.1 (1910)</td>
<td>0.8 (48)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* repeatability = within-run precision  
** intermediate precision = total precision / between-run precision / between-day precision

**cobas c systems**
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).

Sample size (n) = 89

Passing/Bablok

\[ y = 0.993x + 0.158 \text{ mg/dL} \]
\[ r = 0.996 \]

The sample concentrations were between 2.70 and 23.4 mg/dL (161 and 1392 μmol/L).

Sample size (n) = 86

Passing/Bablok

\[ y = 0.997x + 0.456 \text{ mg/dL} \]
\[ r = 0.999 \]

The sample concentrations were between 6.35 and 269 mg/dL (378 and 16036 μmol/L).

References

Appendix N: Dilution curve

The dilution of the plasma with distilled water showed a consistent relationship with uric acid concentrations. This allowed samples that were not large enough for the Cobas c 311 auto-analyser to be diluted without compromising accuracy.