THE EFFECT OF DAIRY PRODUCTS ON SATIETY, FOOD INTAKE AND POST-PRANDIAL GLYCEMIA IN YOUNG AND OLDER ADULTS

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
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ABSTRACT

The hypothesis that dairy consumed with carbohydrate decreases appetite, food intake (FI) and post-prandial glycemia (PPG) compared with non-dairy beverages in young and older adults was explored. Experiment 1 compared 1% milk and soy, almond and yogurt beverages and water consumed with cereal by young adults. Yogurt suppressed appetite and FI was lowest after milk. Despite higher carbohydrate content, dairy beverages did not result in higher glucose than almond and water. Experiment 2 sought to compare different forms of dairy using 2% milk, soy beverage, 2% Greek yogurt and cheese consumed with bread and jam by older adults. Yogurt and cheese suppressed appetite more than milk and attenuated glucose compared to milk and soy with no differences in FI. In conclusion, satiety is higher and glucose is lower after carbohydrates are consumed with dairy as well as non-dairy beverages, but semi-solid or solid forms may be more efficacious than liquids.
Table of Contents

List of Tables……………………………………………………………………………………..vii
List of Figures………………………………………………………………………………viii
List of Abbreviations…………………………………………………………………………ix
List of Appendices…………………………………………………………………………..x

**Chapter 1: Introduction** .........................................................................................1

**Chapter 2: Literature Review** ...............................................................................3

2.1 Introduction ........................................................................................................3

2.2 Relationship between Dairy Consumption, Obesity and Type 2 Diabetes ..........3

2.3 Milk .....................................................................................................................4

2.3.1 Milk Proteins ..................................................................................................4

2.3.1.1 Physiological Effects of Satiety and Post-Prandial Glycemia .................5

2.3.2 Milk Fat ........................................................................................................7

2.3.2.1 Physiological Effects of Satiety and Post-Prandial Glycemia .................8

2.3.3 Milk Carbohydrate .......................................................................................8

2.3.3.1 Physiological Effects of Satiety and Post-Prandial Glycemia .................9

2.3.4 Other Dairy Products ...................................................................................9

2.3.4.1 Cheese ......................................................................................................9

2.3.4.2 Yogurt ......................................................................................................9

2.3.5 Non-Dairy Alternatives ..............................................................................11

2.3.5.1 Soy Beverage ..........................................................................................11
List of Tables

Table 4.1 Nutritional information of treatments and breakfast cereals.................................42
Table 4.2 Energy intake, cumulative energy intake, water intake and caloric compensation......43
Table 4.3 Effect of treatment on post-treatment, post-meal and cumulative mean changes
from baseline for subjective appetite, blood glucose and insulin.................................44
Table 4.4 Effect of treatment on post-treatment, post-meal and cumulative ratios of blood
  glucose to insulin iAUC and on ratios of blood glucose to insulin changes from
  baseline at 30 min........................................................................................................45
Table 5.1 Nutritional information of treatments and breakfast foods.................................62
Table 5.2 Energy intake, cumulative energy intake, water intake and caloric compensation.....63
Table 5.3 Effect of treatment on post-treatment, post-meal and cumulative mean changes from
  baseline for subjective appetite, blood glucose and insulin.................................64
Table 5.4 Effect of treatment on post-treatment, post-meal and cumulative ratios of blood
  glucose to insulin iAUC and on ratios of blood glucose to insulin changes from
  baseline at 30 min........................................................................................................65
Table 6.1a Compliance to Health Canada’s Draft Guidance Document for Satiety..............74
Table 6.1b Compliance to Health Canada’s Draft Guidance Document for the Reduction in
  Post-Prandial Glycemia..............................................................................................75
List of Figures

Figure 4.1. Experiment 1: Subjective appetite changes from baseline in the cumulative period...............................................................46

Figure 4.2. Experiment 1: Blood glucose changes from baseline in the cumulative period........46

Figure 4.3. Experiment 1: Insulin changes from baseline in the cumulative period...........47

Figure 5.1. Experiment 2: Subjective appetite changes from baseline in the cumulative period...............................................................66

Figure 5.2. Experiment 2: Blood glucose changes from baseline in the cumulative period.......66

Figure 5.3. Experiment 2: Insulin changes from baseline in the cumulative period.............67
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>Bioactive Peptides</td>
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<tr>
<td>BCAA</td>
<td>Branched-Chain Amino Acids</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CLA</td>
<td>Conjugated Linoleic Acid</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>EFSA</td>
<td>European Food Safety Authority</td>
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<td>FI</td>
<td>Food Intake</td>
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<td>GI</td>
<td>Glycemic Index</td>
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<tr>
<td>GIP</td>
<td>Glucose-Dependent Insulinotropic Peptide</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-Like Peptide-1</td>
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<td>GMP</td>
<td>Glycomacropeptide</td>
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<td>h</td>
<td>Hour</td>
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<tr>
<td>HC</td>
<td>Health Canada</td>
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<td>IR</td>
<td>Insulin Resistance</td>
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<td>kcal</td>
<td>Kilocalories</td>
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<td>min</td>
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<td>PCR</td>
<td>Protein to Carbohydrate Ratios</td>
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<td>PPG</td>
<td>Post-Prandial Glycemia</td>
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<tr>
<td>PYY</td>
<td>Peptide Tyrosine Tyrosine</td>
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<tr>
<td>RCT</td>
<td>Randomized Clinical Trial</td>
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<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>UHT</td>
<td>Ultra-High Temperature</td>
</tr>
</tbody>
</table>
List of Appendices

Appendix 1. Sample Size Calculation ..................................................................................92
Appendix 2. CONSORT Flow Diagram ..................................................................................93
Appendix 3. Experimental Protocols .................................................................................95
Appendix 4. Beverage Ingredients ......................................................................................96
Appendix 5. Nutritional Composition of Treatments ............................................................97
Appendix 6. Recruitment Materials ...................................................................................98
Appendix 7. Information and Consent Forms ......................................................................100
Appendix 8. Screening Questionnaires ..............................................................................112
  8.1 Primary (Phone/Email) Screening Questionnaires .......................................................113
  8.2 Secondary (In-Person) Screening Questionnaires .......................................................120
  8.3 Sleep and Breakfast Habits Questionnaire ..................................................................129
  8.4 Food Acceptability and Frequency Questionnaire ......................................................130
  8.5 Eating Habits Questionnaire ......................................................................................132
  8.6 Participant Eligibility Summary ..................................................................................133
Appendix 9. Study Day Forms ............................................................................................135
  9.1 Sleep Habits and Stress Factors Questionnaire .........................................................136
  9.2 Recent Food Intake and Activity Questionnaire .......................................................137
  9.3 Motivation to Eat VAS ...............................................................................................138
  9.4 Physical Comfort VAS ...............................................................................................139
  9.5 Energy and Fatigue VAS ............................................................................................140
9.6 Treatment and Test Meal Palatability VAS.............................................141

Appendix 10. Blood Glucose and Insulin Tracking Sheet.................................................142

Appendix 11. Lunch Meal and Water Intake Tracking Sheet.............................................144
Chapter 1
Introduction

Since the 1980s, worldwide obesity has more than doubled [1]. In Canada, 54% of adults were considered overweight or obese based on 2014 self-reported data [2]. These individuals are often also characterized by the metabolic syndrome, which is defined as having at least three of the following: 1) abdominal obesity, 2) elevated plasma triglycerides, 3) elevated fasting blood glucose, 4) decreased HDL cholesterol, and 5) elevated blood pressure. In fact, data from the Canadian Health Measures Survey from 2009-2011 indicated that 22% of adults had metabolic syndrome [3]. Therefore, not only is overweight and obesity amongst the Canadian population estimated to carry an annual economic burden of up to $7.1 billion [4], it is associated with increased risk of co-morbidities such as type 2 diabetes (T2D) [5] for which the risk is increased [6]. As of 2015, 9.3% of adults in Canada were living with diabetes and 22.1% with prediabetes [7]. As a result, consumers are turning to dietary measures and foods that help prevent or manage obesity and T2D [8].

In response to this demand, the food industry and the scientific community have become focused on identifying effective foods and food components to improve these metabolic outcomes. To encourage this development, government regulatory agencies have approved health claims and provided guidance on study designs to be used as evidence to substantiate food health claims. More recently, guidance has been provided to claims related to appetite and blood glucose control. In 2012, the European Food Safety Authority (EFSA) released their document titled “Guidance on the scientific requirements for health claims related to appetite ratings, weight management, and blood glucose concentrations” [9]. Health Canada (HC) also released two draft guidance documents on food health claims for “satiety” and “the reduction in post-prandial glycemic response” in 2012 and 2013, respectively [10, 11]. Still, the feasibility of these guidelines remains to be tested by actual studies.

Dairy products have been identified as functional foods that can aid in obesity and T2D management. Not only do dairy products provide a rich vitamin and mineral profile and an excellent source of high quality protein, epidemiological studies have shown an association
between frequent dairy consumption and lower incidences of obesity and T2D [12, 13]. Randomized clinical trials (RCT) have shown high-dairy diets to be effective on weight loss [14] and beneficial in improving T2D risk factors such as insulin resistance (IR) [15, 16]. The improvements on these metabolic outcomes have been mainly attributed to the dairy proteins, especially whey. Short-term studies investigating the effects of whey have shown that it can increase satiety, and decrease FI and PPG through insulin-dependent and insulin-independent mechanisms [17, 18]. However, there has been little investigation on the effect of dairy products as a whole, consumed in typical serving sizes and as part of a meal, on these outcome measures. Studies of that design are necessary to assess the effectiveness of real life dairy consumption.

Despite the health benefits of dairy products, most Canadians do not consume the two to three servings a day recommended by Canada’s Food Guide [19, 20]. When per capita cheese, yogurt, and milk consumption for 2015 is averaged, Canadians only consumed 1.7 servings of dairy a day. In fact, milk consumption has decreased steadily over the past few decades and in 2015, the per capita consumption was 70.64 L compared to 90.05 L in 1996 [20]. Dairy consumption has also shown to decrease with age with only 26% of men and 20% of women aged 51 to 70 years old consuming the recommended amount in 2004 [21]. This population is vulnerable for developing T2D due to metabolic inflexibility [22] and at risk for developing sarcopenia due to aging [23]. Therefore, inclusion of dairy in their diets could greatly improve and regulate their metabolic and overall health outcomes. However, there have been no studies specifically targeting this age group. Therefore, the focus of this thesis is the effect of dairy products on satiety, food intake (FI) and post-prandial glycemia (PPG) in healthy young and older adults.
Chapter 2
Literature Review

2.1 Introduction

To provide background for this thesis research, the following literature review is composed of seven main sections. First, the relationship between dairy consumption, obesity, and T2D is examined. This is followed by a review of the macronutrient components of milk and their effects on satiety, FI and PPG. Next, a brief overview of the physiological mechanisms regulating satiety, FI and PPG are provided and finally, a current review of the literature regarding the effects of dairy on these outcomes measures.

2.2 Relationship between Dairy Consumption, Obesity, and Type 2 Diabetes

Frequent consumption of dairy products is associated with lower incidences of obesity [12, 13] and T2D [13]. Data collected across multiple large prospective cohort studies show that long-term weight gain is inversely related to dairy consumption [24-26]. It has also been observed that in both obese and normal-weight individuals, as the number of daily servings of dairy increase there is a smaller increase in waist circumference, percent body fat [25, 27] and body mass index (BMI) [28]. As a result, there have been many RCTs studying the effect of dairy consumption as a dietary tool for weight loss. Evidence from these RCTs indicate that dairy consumption may aid with weight loss in the short-term or when individuals are on energy-restricted diets but its effects in the long-term are still unclear [14].

Similarly, large prospective cohort studies have also reported an inverse relationship between dairy consumption and risk of T2D [29]. The incidence of IR [26], and other markers of glycemia and insulinemia [30] have shown to be improved with more frequent dairy consumption. A 6-month RCT with a crossover design compared a high-dairy (4 servings/day) and a low-dairy (≥2 servings/day) diet in healthy participants and found that increasing dairy consumption improved measures of IR [15]. It has been proposed that dairy positively affects these health outcomes through its ability to promote satiety, reduce FI and PPG.
2.3 Milk

Milk can be described as an emulsion of fat globules dispersed within a water-based fluid containing carbohydrates, proteins, vitamins and minerals [31]. Since it is the sole source of nutrition for the mammalian young, it is an extremely rich source of nutrition [32]. Cow’s milk, hereby simply referred to as milk, is the most widely consumed form of animal milk by humans. On average, the composition of milk is 87% water, 4% to 5% carbohydrate, 3% protein, 3% to 4% fat, 0.8% minerals, and 0.1% vitamins [33]. It contains both water- (B complex, C) and fat-soluble (A, D, E) vitamins as well as the minerals phosphorus, magnesium, zinc, selenium, and calcium. In addition to its rich micronutrient profile, milk proteins are considered one of the highest quality proteins since it is an excellent source of essential amino acids [34]. In fact, it is the milk proteins that are thought to be largely responsible for the effects on satiety, FI and PPG.

2.3.1 Milk Proteins

Milk proteins are composed of 80% casein and 20% whey protein, with each made up of a unique combination of protein sequences containing bioactive peptides (BAP) that exert different physiological effects [35, 36]. Casein consists of four major subunits: \(\alpha_{s1}\), \(\alpha_{s2}\), \(\beta\), and \(\kappa\)-caseins, with each containing BAP that can be classified into opioid, antihypertensive, immunodefensing, antithrombic, antioxidative, and mineral-binding peptides [36]. These BAP, which exert hormone-like activities, are activated by enzymatic hydrolysis that occurs in the stomach by digestive enzymes (e.g. trypsin or pepsin) or by food processing such as bacterial fermentation in cheese- and yogurt-making. In cheese-making, glycomacropeptide (GMP) is formed by the cleavage of \(\kappa\)-casein by rennet (chymosin) and along with whey is a by-product of the cheese-manufacturing process. Therefore, when whey is further processed into isolates and concentrates, GMP often makes up 20-25% of the total protein concentration [37]. Naturally, whey consists of \(\alpha\)-lactalbumin, \(\beta\)-lactoglobulin, serum albumin, immunoglobulins, and lactoferrin, that contain BAP that can exhibit similar physiological activities as those derived from casein [38]. Whey is also extremely rich in branched-chain amino acids (BCAA), especially leucine.

Due to differences in their chemical and physical structure, whey and casein differ in their rate of digestibility. Whey is considered a “fast” and casein a “slow” protein based on the rate of protein hydrolysis and the rate of appearance of amino acids in the plasma following ingestion [35].
When whey is ingested, peak plasma amino acids are reached 40 min to 2 h later and return to baseline within 3 to 4 h, whereas casein results in a slower and more sustained release of amino acids that returns to baseline up to 7 h later [39]. This occurs because whey is a soluble protein that is quickly digested and absorbed, whereas casein clots in the stomach to form a gel which results in slower gastric emptying [40].

2.3.1.1 Physiological Effects on Satiety and Post-Prandial Glycemia

Although the precise mechanisms by which milk promotes satiety and decreases PPG are still unclear, the effects have been mainly attributed to the amino acids in the milk proteins and the physiological properties of their BAP. Their BCAA-rich profile has been offered as a main explanation. Since the brain uses BCAAs as fuel, an increase in BCAA in the blood may signal satiety in the brain in a manner similar to glucose [35]. More specifically, whey is extremely rich in leucine which enters the brain quicker than any other amino acid and has been shown to suppress FI [41].

Whey is known as a potent stimulator of insulin and its BCAAs have been identified as the main insulin secretagogue component [42]. When free BCAAs and intact whey were consumed, the insulin response following the free BCAAs was lower than after whey [43]. This difference can be explained by the lower levels of glucose-dependent insulinotropic polypeptide (GIP) observed after ingestion of the free BCAAs compared to whey. GIP is a gastrointestinal hormone belonging to the incretin family, which are potent insulin stimulators. This suggests that the insulinotropic effect of whey is in part due to the incretin hormones that are stimulated by BAP unlocked during digestion rather than free amino acids [43]. Milk proteins are also potent stimulators of other gastrointestinal hormones that slow gastric emptying to signal satiety and attenuate PPG [35, 44]. In fact, there is evidence that whey attenuates PPG through insulin-dependent and insulin-independent mechanisms [18].

Many studies have shown milk proteins capable of increasing satiety but this is not always predictive of reduced FI at a later meal. A study in overweight and obese women comparing whey preloads (5, 10, 20 g) to a water control found all whey preloads increased satiety but did not lead to reduced FI 2 h later [45]. In another study in healthy young adults, 25% energy from casein increased satiety more than 10% but there were no differences in FI 3 h later [46]. In the
same study, although 10% energy from whey increased satiety more than the same amount of casein and soy protein, FI was the same. The lack of correlation between satiety (as measured by subjective appetite ratings) and FI may be explained by differences in protein source, quantity and time to the next meal [35]. This is supported by studies with shorter times between preload and test meal and those comparing whey to other protein sources such as tuna, turkey and egg albumin and non-protein sources such as glucose [39, 47-50].

There is no consistent evidence supporting one milk protein to be more satiating than the other [46, 51, 52]. When appetite ratings were measured up to 180 min following consumption, whey was more satiating than casein whereas when ratings were measured to 330 min, the opposite was seen [53, 54]. This indicates that whey is more satiating in the short-term, and casein, in the long-term, which can be explained by whey’s “fast” and casein’s “slow” rate of digestibility. However, consuming milk proteins in combination may be more effective for increasing satiety and reducing FI than consuming either one alone. In a comparison of three isocaloric beverages of whey, casein and skim milk (whey and casein), skim milk reduced FI at the later meal more than either whey or casein alone [55]. Another study found a combination of whey and casein increased cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), two gastrointestinal hormones, more than whey alone [44].

Consumption of milk has been shown in several studies to not only promote satiety but also reduce later FI [55-57]. In one study, 240 mL of low-fat (1.5%) milk increased satiety and reduced FI more than the same amount of apple juice in obese boys [56]. This is especially important because it showed that consuming milk in a typical serving size (e.g. 250-500 mL) is of functional significance to satiety and FI. Often, the amount of milk proteins tested in studies is unattainable by typical dairy consumption. For example, studies have tested up to 50 g of milk proteins using isolated protein powders [39] whereas one serving of milk (250 mL) only has 9 g.

Investigations of the effects of milk on glycemia have mainly focused on the effects of whey. Many studies have shown that whey consumed prior to, or along with carbohydrate leads to lower PPG than carbohydrate alone [18, 47, 58-60]. In healthy young adults, preloads of 10-40 g of whey resulted in lower PPG following an ad libitum pizza meal consumed 30 min later compared to the water control [48]. Another study in healthy young adults showed that 9 and 18 g of whey, consumed with 25 g of glucose, led to lower PPG than glucose alone [59]. The 18 g
whey preload also increased insulin more than the glucose preload. Similar results were seen in T2D individuals when they consumed 50 g of whey immediately followed by a high-GI breakfast. Compared to the water control, whey lowered the PPG response by 28%, increased insulin by 105% and total GLP-1 was 141% higher following the whey preload over 3 h [58]. Therefore, the attenuation of PPG by whey has often been solely attributed to an increase in insulin, mediated by stimulation of insulin by incretin hormones and BCAAs.

However, insulin-independent mechanisms also play a role in reduction of PPG including delay of gastric emptying. When 10 and 20 g of whey were consumed, lower PPG and slower gastric emptying, as measured by plasma paracetamol concentrations, were seen compared to 10 g of glucose [18]. In another study, 25 g of whey protein also slowed gastric emptying, as measured by scintigraphy and reduced PPG compared to 25 g of glucose [61]. Similarly a study that compared three Greek yogurts, skim milk and orange juice with varying protein to carbohydrate ratios (PCR) found improved PPG following the dairy preloads with no differences in insulin responses between the treatments [62]. Still, more studies that include insulin as an outcome measure are needed to fully describe insulin-independent attenuation of PPG by milk proteins.

2.3.2 Milk Fat

Raw milk is usually composed of 4% fat although the quality and composition of fat may be influenced by many factors such as season, breed, age, nutritional status and diet of the cow, time of milking, stage of lactation [63]. Milk fat consists of 98% triglycerides while other milk lipids are diacylglycerol (~ 2% of the lipid fraction), cholesterol (< 0.5%), phospholipids (1%) and free fatty acids (0.1%). Milk triglycerides contain nearly 400 different types of fatty acids, making milk fat the most complex of all natural fats. Approximately 70% of milk fat is saturated, 25% monounsaturated, 2.3% polyunsaturated and 2.7% trans [64].

Milk fats have traditionally been associated with cholesterol-raising effects due to its high long-chain saturated fatty acid content [65] thus leading to the emergence of fat-free and low-fat dairy products. However, a review of epidemiological data has not found any link between consumption of dairy and cardiovascular disease (CVD) [66]. In fact, a review investigating the effect of high-fat dairy and CVD found that in 11 out of 16 observational studies, high-fat dairy was inversely related to adiposity which is a risk factor for CVD [67]. It is hypothesized that
similar to milk proteins, milk fats contain bioactives such as conjugated linolenic acids (CLA) [68] that improve metabolic health outcomes and also play a role in the regulation of satiety, FI and PPG.

2.3.2.1 Physiological Effects on Satiety and Post-Prandial Glycemia

Milk fat may contribute to satiety and reduced FI through regulation of gastric emptying and gastrointestinal hormones [69]. Studies have shown that triglycerides or fatty acids entering the small intestine reduces hunger and impairs FI. This may be explained by the increase in gastrointestinal hormones such as CCK and peptide tyrosine tyrosine (PYY) that is observed following fat in the duodenum. However, studies suggest that the satiating effect from fat entering the ileum is also due to the ileal brake phenomenon which is a mechanism by which gastric emptying is slowed. Chain length and degree of saturation are both factors that determine the magnitude of effect of a fat [70].

Currently, there are no studies that have compared the effects of fat-free, low- and high-fat dairy products on satiety and FI. However, there have been studies testing the effects of specific fatty acids that are contained within milk fats. A study in overweight men and women compared the effects of 1.8 g or 3.6 g of CLA, or a placebo every day for 13 weeks on satiety and FI [71]. After the 13-week intervention period, both CLA treatments resulted in increased feelings of satiety suggesting that fat plays a role in promoting satiety.

A previous study compared the GI and insulin index of whole and skimmed milk and found no differences [72]. Additionally, skim milk has been shown to reduce PPG [62]. Still, few studies have compared milks of different fat contents consumed in typical serving sizes and as part of a meal and there may be enhanced PPG control and or longer duration in satiety if full-fat milk was consumed.

2.3.3 Milk Carbohydrate

Lactose, a disaccharide composed of glucose and galactose, is the main source of carbohydrate in milk. It is found in the water-based fluid of milk and is contained within the whey. Lactose is essentially unique to milk aside from small amounts being identified in the fruit of some plants. Aside from those individuals that are lactose intolerant, it is readily digested in humans by the
enzyme lactase. There are also small amounts of other carbohydrates in milk such as glucose, galactose and oligosaccharides.

2.3.3.1 Physiological Effects on Satiety and Post-Prandial Glycemia

Lactose produces a lower glycemic response than sucrose or glucose, which may contribute to the low GI of milk [42]. When lactose and whole milk were compared (both containing 25 g of lactose), whole milk resulted in a lower PPG response which indicates an additive effect of the lactose and protein [73]. This is further evidenced by a recent study that found that when whole milk and a lactose-only beverage (both containing 24 g of lactose) were compared, whole milk resulted in lower PPG [74]. When 56 g of lactose and glucose were compared in overweight men, FI was lower after lactose at 180 min later [75].

2.3.4 Other Dairy Products

Milk is used to make a variety of dairy products that differ in their processing, macronutrient content, protein ratio and food form. As a result, products like yogurt and cheese may affect satiety, FI and PPG differently than milk.

2.3.4.1 Cheese

In general, cheese is made by the coagulation of casein through the addition of the enzyme rennet. The solid curds are pressed together to form the final product while the liquid whey fraction is removed. There are hundreds of varieties of cheeses ranging in macronutrient content and texture (i.e. hard, semi-soft, soft) due to different processing techniques. Typically, cheese is very low in carbohydrate content due to the removing of lactose with the whey and is high in protein and fat content.

2.3.4.2 Yogurt

Yogurt is made by the fermentation of milk with bacterial cultures and the resulting product is semi-solid with a gel-like consistency. There are two main types of yogurt: stirred, which is known as regular yogurt and strained, which is commonly referred to as Greek yogurt and has a much thicker and creamier consistency. Regular yogurt is comparable to milk (of the same fat
percentage) in terms of macronutrient content and whey to casein ratio. However, the macronutrient content of yogurts may vary due to additives, such as sweetening agents that increase carbohydrate content and added milk powder that may increase protein content [76], and degree of processing. For example, drinkable yogurt beverages are often high in added sugars and therefore lower in protein. Greek yogurt is made by straining out the liquid whey fraction that is kept in regular yogurt. This results in a more concentrated product that is low in lactose and has a higher protein density (approximately double than the same serving of regular yogurt) that is comprised mainly of casein. It is important to note that the processing of milk to yogurt (i.e. heating and fermentation processes) can change the structure and nature of certain macronutrients. For example, yogurt is found to have a higher concentration of CLA than milk and the denaturing of whey is shown to occur.

Although few studies have investigated the effect of yogurt on satiety and FI, there is evidence it is satiety-promoting although its effect on FI has not been supported. A study compared a drinkable yogurt (10.2 g protein) to chocolate bars (2.7 g protein) and found that although yogurt induced higher satiety than the chocolate bars over the first 60 min, it did not result in delayed requests for the next meal and reduced FI [77]. Conversely, a study comparing common snacks found a drinkable yogurt (5.2 g protein) to increase satiety and reduce FI more than water and crackers (2.4 g protein) [78].

Comparing food form was the objective of a study that tested a semi-solid peach yogurt (17.1 g protein), the same yogurt in liquid form (17.1 g protein), a peach-flavoured dairy beverage (2.6 g protein) and peach juice (0 g protein) [79]. Both yogurts were more satiating than the other preloads but did not result in reduced FI. There were no differences between the semi-solid and liquid yogurts indicating in this case that food form was not an important determinant of satiety when macronutrients were identical. The effect of protein was tested in a study that compared yogurts with low (5 g), moderate (14 g) and high (24 g) protein contents [80]. The high protein yogurt led to increased satiety and later request for the next meal compared to the other yogurts. However, no differences in satiety and FI were seen when Greek yogurts (23.1 g, 22.2 g, or 18.3 g protein), skim milk 16.6 g protein) and orange juice (2.4 g protein) were compared [62]. In the same study, it was found that the Greek yogurts and milk attenuated PPG more than orange juice in a protein-dependent manner.
Only one study has reported a comparison of the effects of yogurt, cheese and milk on satiety, FI and PPG. Isocaloric (841 kcal) amounts of yogurt (10.9 g protein, 15.9 g carbohydrate), semi-skimmed milk (13.9 g protein, 20.4 g carbohydrate) and Cheddar cheese (12.3 g protein, 0 g carbohydrate) were consumed by healthy men followed by an ad libitum meal served 90 min later [81]. Hunger was 8 and 10% lower and FI was 11 and 9% lower after yogurt than cheese and milk, respectively. There were no differences in glycemic response or gastrointestinal hormones. This study indicates that when comparing different types of dairy, macronutrient content may not be a main determinant of satiety and FI due to differences in protein ratios, physical form, processing techniques (e.g. bacteria from fermentation) and energy density that may affect physiological functions like gastric emptying differently. Still, more studies are needed to provide a better understanding on the differences between different dairy products.

2.3.5 Non-Dairy Alternatives

Due mainly to the perceived negative views towards the healthfulness of cow’s milk and the increased activism towards animal welfare (e.g. veganism) [82], there has been a strong emergence of non-dairy alternatives onto the market over the last decade. In 2015, sales of non-dairy beverages in the United States grew by 9% to reach $1.9 billion USD while sales of milk dropped by 7% [83]. Sales of non-dairy beverages are estimated to grow 47% between 2013 and 2018 [82]. Although the popularity of non-dairy beverages began with soy beverages, almond beverages have surpassed them in popularity. Data from the United States shows that in 2014, almond beverages accounted for 60% of non-dairy beverage sales while soy beverage only captured 30% of the market [84]. Commercially-available non-dairy beverages often have added calcium and vitamin D to match the vitamin content of milk as well as gelling and thickening agents such as carrageenan and gellan gum. In Canada, labelling regulations permit the word “milk” to only refer to cow’s milk and cannot be used to refer to non-mammary sources such as plant-based milk alternatives [85]. Since the emergence of these plant-based milk alternatives is still relatively recent, minimal research has been conducted on their effects on satiety, FI and PPG.
2.3.5.1 Soy Beverage

Soy beverage, often referred to as “soy milk”, is a milk substitute derived from the processing of soybeans. Traditionally, it is made by soaking soybeans overnight and then grinding them into a slurry. Cold water is then added and mixed thoroughly into the slurry before the mixture is strained and pressed through cheesecloth to remove large solids. The remaining liquid is then boiled and strained again. The resulting product is a heavy and gritty liquid with a strong bean flavour and chalky mouthfeel that is not generally acceptable to Western palettes [86]. Today, commercial manufacturing of soy beverage includes processes to make the product more palatable such as blanching the soybeans to inactive bitter tasting enzymes, decanting to remove insoluble fibres to avoid chalkiness and adding sweeteners and flavourings. Technologies common to milk processing such as ultra-high temperature (UHT) sterilization have also been adopted [87].

There is evidence from epidemiological and animal and human intervention studies to suggest a beneficial effect of soy on obesity and T2D risk factors [88, 89]. Like dairy, the effects have been mainly attributed to the protein component of the soybeans [89, 90]. Since soy is a complete protein with a high concentration of BCAAs, it is considered one of the highest quality plant protein sources [91]. Still, its nutritional quality is considered slightly lower than that of milk proteins [92]. In terms of rate of digestion, soy protein is considered a “fast” protein comparable to whey. Therefore, when soy and mixed milk proteins were compared, soy resulted in a quicker intestinal transit time likely due to the delayed gastric emptying properties of casein [93]. One serving (250 mL) of commercially-available soy beverage usually contains slightly less protein (1-4 g less) than one serving of milk but this is dependent on the products compared.

A few short-term studies show that soy protein, consumed in isolated powder form in a beverage, promotes satiety and reduces later FI [51, 54] and PPG [35]. When soy protein is compared to milk protein on satiety and FI, the results have not been consistent. In one study, soy was found to be equally satiating as casein and more than whey [54], whereas another found whey to be more satiating than soy [46]. Still, no differences in FI at a meal following consumption of soy protein and whey have been observed [39, 51].
When 500 mL of 2% milk (260 kcal, 18 g protein, 24 g carbohydrate) and soy beverage (200 kcal, 14 g protein, 16 g carbohydrate) were compared, there were no differences in satiety or FI [94]. There were also no differences in PPG in the post-treatment period but milk resulted in lower PPG in the post-meal (140-260 min). Another study compared the effects of soy beverage and 1% milk when consumed with high GI carbohydrate [95]. Isovolumetric (322 mL) and isocaloric (311 kcal) amounts of soy beverage and 1% milk both containing 17.6 g protein were either fed 30 min prior to consuming white bread (50 g carbohydrates) or consumed together with the white bread as part of a meal, all which were compared to white bread alone. All treatments led to lower blood glucose than white bread alone but preloading treatments resulted in lower blood glucose for 120 min than co-ingestion. There were no differences between soy beverage and 1% milk when co-ingested but 1% milk resulted in lower blood glucose when preloaded. Soy beverage resulted in higher insulin than 1% milk when co-ingested and preloaded. Similarly, no differences in PPG between whey and soy protein however greater insulin and GLP-1 concentrations were observed following whey. This indicates that soy does not have the same insulinotropic potency as whey [46]. In the same study, casein attenuated PPG more than soy.

2.3.5.2 Almond Beverage

Almond beverage, often referred to as “almond milk” is a milk substitute derived from the processing of almonds. Very similar to soy beverage, almond beverage is made by blending soaked raw almonds with water and then straining out large solid fractions [96]. Technologies like UHT sterilization and high pressure processing are also employed in almond beverage production.

Many studies have found an association between almond consumption and lower risk of obesity and T2D [97-99]. This has been attributed to the rich nutrient profile of almonds since they are high in unsaturated fats, dietary fibres and protein [97]. However, the majority of commercial almond beverages do not contain the same nutritional composition as whole almonds for various reasons related to processing. In fact, one serving (250 mL) of almond beverage usually only contains approximately 1 gram of protein which is equivalent to 3 whole almonds. Currently, the
correlation between almond beverages and obesity and T2D risk factors has not been investigated and there are no studies testing its effects of satiety, FI and PPG.

2.4 Regulation of Satiety and Food Intake

The concept of satiety is of interest as a strategy to combat the obesity epidemic. Satiety is defined as the feeling of fullness after an eating occasion. The magnitude and length of time for which satiety is achieved may dictate timing and/or amount of subsequent FI. Increasing satiety may inhibit or delay the next eating occasion and/or reduce subsequent FI, which may lead to a reduced daily energy intake. Over time, repeated reductions in energy intake may aid with weight loss and weight maintenance. Therefore, much research has been conducted on determining the satiating effects of foods for the use of providing useful and evidence-based diet guidelines and recommendations.

It has been established that of the three macronutrients, protein is more satiating than fat and carbohydrate [100]. Still, this knowledge alone is not adequate because there are many different types of protein (e.g. plant vs. animal) that each have different satiating properties. Additionally, since we rarely ingest macronutrients in isolation, it is important to understand how different macronutrient properties within a food and a meal can interact to affect satiety. However, in order to study a food’s satiating potential, it is necessary to first understand the physiologies that affect satiety.

Satiety is the result of a multitude of nutrient, neuronal and hormonal signals that converge in the brain to control appetite and FI [101]. These processes can be categorized into pre-absorptive (i.e. post-ingestive) and post-absorptive stages that respectively determine the early and late stages of satiety. It is important to note that many of the processes that determine early satiety are those that lead to satiation. Unlike satiety, satiation refers to the feelings within an eating occasion that lead to meal termination and dictate meal size. To a certain extent, the same physiological processes that lead to satiation persist to determine the magnitude and duration of satiety. There are also sensory and cognitive factors that determine satiation and early satiety however, that is beyond the scope of this thesis and the discussion that follows will only focus on the physiological mechanisms of pre- and post-absorptive stages.
2.4.1 Gastric Distension and Emptying

Gastric distension plays a key role in satiation and satiety. As food is ingested, gastric accommodation occurs (i.e. expansion of the stomach). This triggers tension and stretch mechanosensitive receptors in the gastrointestinal tract to send neuronal signals via vagal and splanchnic nerves to the brain to signal satiation [102]. Studies have shown that inflating a balloon in the stomach [103] and delaying gastric emptying results in increased feelings of fullness and satiety [104]. Therefore, foods that increase magnitude and duration of gastric distension by delaying gastric emptying should increase satiety in the early stages. Gastric emptying rate is dependent on food properties, such as viscosity, and nutrient and caloric content. Foods with high viscosity (e.g. solids) result in a slower emptying rate than foods with low viscosity (e.g. liquids) and the same inverse relationship has been observed with caloric and nutrient content [105].

2.4.2 Gastrointestinal Hormones

As gastric emptying begins, gastrointestinal hormones play a significant role in satiety. As the digested food enters the small intestine, the nutrient composition of the meal is sensed and hormones are released. Gastrointestinal hormones can be divided into two categories: orexigenic and anorexigenic. Anorexigenic hormones, or appetite-inhibiting hormones, increase following a meal to inhibit FI and increase satiety by mechanisms such as decreasing gastric emptying rate to allow for adequate absorption of nutrients [102]. Conversely, orexigenic hormones such as ghrelin are appetite-stimulating and decrease following FI. Anorexigenic hormones include CCK, PYY and GLP-1. CCK is known to be stimulated by the presence of fat (i.e. long-chain fatty acids) and protein (i.e. amino acids) in the duodenum, GLP-1 by the presence of fat and carbohydrate (i.e. simple sugars) in the ileum and PYY by all macronutrients in the colon [106]. Still, studies have shown each macronutrient stimulates each hormone to a different extent which may explain why protein is most satiating[107]. Even within one macronutrient, different sources may exert different effects. In one experiment, plasma CCK and GLP-1 levels were higher after a preload of whey than casein [53]. Therefore, foods with higher potency for stimulating the anorexigenic hormones and/or suppressing the orexigenic hormones may lead to increased and prolonged satiety.
2.4.3 Glucose and Insulin

There is evidence that elevated levels of glucose and insulin following a meal act as satiety signals to the brain. The glucostatic theory proposes that glucoreceptors in the brain sense plasma blood glucose concentrations to regulate feelings of appetite and FI such that a decrease in glucose causes meal initiation and an increase, meal termination [108, 109]. However, when participants were administered glucose intraduodenally, hunger ratings were lower and satiety ratings were higher than when it was administered intravenously [110]. Infusion of octreotide, a known inhibitor of insulin and gastrointestinal hormone release, reversed the satiating effects of the intraduodenal glucose. This indicates that glucose affects satiety not through absolute blood glucose concentrations, but through stimulation of glucoreceptors in the small intestine that sends signals through the vagal nerve and/or through the release of insulin and incretin peptides. This is further supported by a recent study that found intravenous infusions of glucose did not affect feelings of appetite despite increased plasma blood glucose concentrations [111]. Increased plasma insulin concentrations have also been implicated in increasing satiety and decreasing FI in the short-term by crossing the blood brain barrier and acting as a feedback signal to the brain [112, 113].

2.5 Regulation of Post-Prandial Glycemia

PPG refers to an individual’s plasma blood glucose concentrations following an eating occasion. Elevated PPG beyond homeostatic values is an indication of impaired insulin release by the pancreatic β-cells in response to blood glucose and also decreased insulin sensitivity of peripheral and hepatic systems (i.e. IR) [114]. Impaired β-cell function and IR are the pathogenesis of T2D. The GI is a tool that was developed to rank different carbohydrate-containing foods on its effects on PPG, with high GI foods leading to higher PPG relative to low GI foods [115]. There are epidemiological data that correlate high GI diets with a higher risk of developing T2D compared to low GI diets [116]. This indicates that foods leading to lower PPG may help decrease the risk of T2D in healthy individuals and help with glycemic control to prevent further complications for at-risk individuals and existing patients. However, it is important to consider that macronutrients are rarely consumed in isolation and the GI alone is not necessarily a good predictor of PPG. Co-ingestion of carbohydrate with fats and proteins has
been shown to reduce PPG compared to carbohydrate alone [117, 118]. Therefore, much research has been focused on determining the effect of different macronutrient sources and foods on PPG when consumed alone and as part of a high GI meal. However, it is necessary to understand the factors that affect PPG in order to predict which foods may be beneficial.

2.5.1 Gastric Emptying

Gastric emptying rate is one determinant of PPG. It has been shown that in both healthy and diabetic individuals, slower gastric emptying rates result in lower PPG [119]. As mentioned in the previous section, gastric emptying rate is influenced by a multitude of factors such as the viscosity and physical properties of the food and signalling from gastrointestinal hormones. Decreasing the gastric emptying rate is beneficial because it results in a slower and more gradual exposure of glucose into the small intestine, subsequently leading to slower absorption into the bloodstream. However, studies that have infused glucose directly into the duodenum have shown the rate of infusion is not always directly proportional to PPG (i.e. 2, 3 and 4 kcal/min resulted in the same PPG) [119]. This can be explained by the compensatory action of the incretin hormones.

2.5.2 Incretin Hormones and Insulin

Following ingestion of carbohydrate, insulin is released when the pancreatic β-cells sense an increased level of glucose in the circulation. Insulin works to lower plasma glucose and return the levels to a homeostatic state. Aside from glucose-mediated insulin release, incretin hormones play a major role in stimulating insulin secretion to regulate PPG [120]. In fact, it is thought that incretin hormones are responsible for approximately two thirds of insulin release. Amongst the incretin hormones, GIP and GLP-1 have attracted the most attention. The insulinotropic effect of GIP has been demonstrated in animal studies where mice with a deletion of the GIP receptor gene become glucose intolerant [121] and in human studies where infusion of intravenous GIP along with glucose increased insulin secretion and improved PPG [122]. GLP-1 exerts one of the most potent insulinotropic effects. Not only does it bind to pancreatic β-cells and activate pathways that lead to insulin secretion [123], it also stimulates all steps of insulin biosynthesis and gene transcription leading to increased amounts of insulin. For individuals with impaired glucose tolerance or T2D, GLP-1 may help to attenuate PPG by increasing insulin release.
Therefore, foods and macronutrients that are capable of causing a potent incretin effect may be beneficial for glycemic control.

2.6 Dairy in the Regulation of Satiety, Food Intake and Post-Prandial Glycemia

Epidemiological data has shown that frequent dairy consumption is associated with healthier body composition and body weight and lower risk of T2D. Dairy’s ability to increase satiety and help in FI regulation and decrease PPG have been suggested as explanations for these positive health outcomes. The milk proteins, particularly whey, have been pinpointed as the component eliciting these beneficial effects. As a result, the majority of RCTs in this field have been focused on testing the protein component (using isolated protein powders) which is unreflective of actual dairy consumption. Therefore, there is a lack of studies investigating the effects of dairy products as a whole, consumed in typical serving sizes and as part of a meal, on satiety, FI and PPG.

2.6.1 Satiety and Food Intake

There is evidence that milk consumed as a whole as part of a meal or alone, increases satiety but the effects on later FI are still unclear. A study involving overweight and obese adults (n=13M, 21F; 55.1 y; BMI 32.4 kg/m$^2$) comparing isovolumetric (600 mL) and isocaloric (1062 kJ) amounts of skim milk (25 g protein) and fruit drink (<1 g protein) consumed with a standardized breakfast, found that skim milk resulted in higher ratings of fullness and satisfaction, lower ratings of prospective consumption and lower energy intake at a meal served 4 h later [57]. Similarly, a recent study that compared milk to apple juice and water also found that milk resulted in the lowest total appetite scores (higher satiety) and energy intake at lunch [56]. In this study, obese boys (n=34; 11.14±0.8 y; BMI 27.62±2.7 kg/m$^2$) consumed a standardized breakfast along with isovolumetric (240 mL) amounts of 1.5% milk (401.24 kcal, 19.08 g protein), apple juice (411.44 kcal, 11.276 protein) and water (297.74 kcal, 10.931 protein) in a randomized order followed by a lunch served 5 h later. However, although other studies observed similar results for satiety it did not translate into lower FI. When isovolumetric (500 mL) and isocaloric (215 kcal) amounts of chocolate milk (12.6 g protein) and cola (0 g protein) were compared in healthy young men (n=22; 23±1.8 y; BMI 22.2±1.5 kg/m$^2$), chocolate milk resulted in higher satiety but there were no differences in FI at a meal served 30 min later [124]. Another study in overweight
and obese adults (n=12M, 12F; 33.5 ± 9.2 y; BMI 31.4±3.11 kg/m\(^2\)) testing the same amount of semi-skimmed milk (950 kcal, 17 g protein), cola (900 kcal, 0 g protein) and diet cola (7.5 kcal, 0 g protein) produced the same results for satiety and FI, with the test meal served 4 h later [125]. GLP-1 and GIP following milk was 31% and 45% higher than cola, supporting the mechanism that milk mediates satiety through gastrointestinal hormones. It is possible that the two aforementioned did not produce significant results with FI because the number of participants involved did not provide them with adequate statistical power for that outcome measure.

There is also evidence to support that in the short-term, satiety and FI may be determined by caloric content of the preloads in addition to macronutrient composition. When healthy young adults (n=16M, 13F;22.4±0.4 y; BMI 21.9±0.3 kg/m\(^2\)) consumed isovolumetric (500 mL) amounts of chocolate milk (340 kcal, 18 g protein), 2% milk (260 kcal, 18 g protein), soy beverage (200 kcal, 14 g protein), infant formula (368.2 kcal, 7.6 g protein) and orange juice (229 kcal, 4 g protein), only chocolate milk and infant formula led to lower FI at a meal served 30 min later [94]. Still, few studies investigating milk have tested preloads in the manner of the aforementioned study using commercially-available products in typical serving sizes.

2.6.2 Post-Prandial Glycemia

There are even fewer studies that have investigated the effects of milk as a whole on PPG. Our group published a study that compared the effects of whole milk to its individual macronutrient components in healthy males (n=12; 22.4±0.4 y; BMI 21.9±0.3 kg/m\(^2\)) [74]. The five treatments consisted of isovolumetric (500 mL) preloads of 3.25% milk (16 g protein, 24 g lactose, 16 g milk fat), lactose (24 g), milk fat (16 g), complete milk protein (16 g) and a simulated milk beverage (16 g protein, 24 g lactose, 16 g milk fat). Whole milk and simulated milk led to the same PPG responses which were 56% lower than the sum of the glycemic responses for each macronutrient (expressed as area under the curve). Therefore, the results of this study highlight the importance of testing milk as a whole entity since macronutrients interact to exert a more potent effect on attenuating PPG.

Currently there is a lack of studies that have tested the effects of milk, consumed in typical serving sizes and as part of a high GI meal on PPG in order to reflect real life consumption. One
study showed that both preloading 322 mL of 1% milk 30 min before consuming white bread and consuming 1% milk together with white bread lowers PPG more than white bread alone [95]. Additionally, in one study, healthy young adults (n=15M, 14F; 22.1±0.4 y; BMI 22.3±0.3 kg/m²) consumed ad libitum amounts of water, 1% milk (110 kcal, 12 g carbohydrate, 9 g protein/serving), regular cola (110 kcal, 30 g carbohydrate, 0 g protein/serving), diet cola (0 kcal, 0 carbohydrate, 0.1 g protein/serving) and orange juice (110 kcal, 24.5 g carbohydrate, 2.1 g protein/serving) along with an ad libitum pizza meal. Despite no differences in fluid volumes between milk and other treatments, milk led to the lowest PPG [126]. There is also evidence that milk can attenuate PPG both post-treatment and post-meal. Post-treatment is defined as the period after treatment consumption and before the test meal and post-meal is defined as the period after the test meal. Isovolumetric (500 kcal) amounts of water, chocolate milk (340 kcal, 56 g carbohydrate, 18 g protein), 2% milk (260 kcal, 24 g carbohydrate, 18 g protein), soy beverage (200 kcal, 16 g carbohydrate, 14 g protein), infant formula (368.2 kcal, 39.6 g carbohydrate, 7.6 g protein) and orange juice (229 kcal, 54 g carbohydrate, 4 g protein) were consumed 120 min before an ad libitum meal [94]. Despite having higher carbohydrate content, chocolate milk resulted in lower PPG than orange juice in the post-treatment (0-120 min) which can be attributed to its higher protein content. In the post-meal (140-260 min), despite no differences in FI at the ad libitum meal, milk resulted in the lowest PPG. This supports the concept of a “second-meal effect” wherein the glycemic response of a later meal is still affected by the preload consumed X amount of time earlier. This “second-meal effect” is also seen in another study that compared preloads of different protein to carbohydrate ratios (PCR) on PPG. Healthy adult males (n=12; 23.4 ± 0.57 y; BMI 21.3±1.15 kg/m²) consumed equal amounts (250 g) of plain yogurt (141 kcal, 10.0 carbohydrate, 23.1 g protein – 2.30 PCR), plain yogurt with honey (165 kcal, 17.9 g carbohydrate, 22.2 g protein – 1.24 PCR), strawberry yogurt (167 kcal, 23.3 g carbohydrate, 18.3 g protein – 0.79 PCR), skim milk (166 kcal, 23.9 g carbohydrate, 16.6 g protein – 0.69 PCR) and orange juice (141 kcal, 32.9 g carbohydrate, 2.4 g protein – 0.07 PCR) and were served an ad libitum meal 120 min later [62]. Post-treatment (0-120 min), PPG was attenuated in a PCR-dependent manner with plain yogurt resulting in the lowest PPG and orange juice, the highest. However, in the post-meal (120-260 min), although all dairy treatments still led to lower PPG than orange juice, skim milk attenuated PPG the most out of all treatments.
2.7 Summary and Research Rationale

It is clear through epidemiological data and RCTs that the health benefits of dairy exceed that of providing basic micro- and macronutrient needs. Through various physiological mechanisms, dairy consumption impacts the regulation of satiety, FI and PPG supporting the hypothesis that it is an important functional food to use in the battle against obesity and T2D. Yet, few studies have examined the role of dairy products when most often consumed at usual meals.

Breakfast is a typical meal when dairy products are consumed with high GI foods (e.g. milk with cereal) [127]. Therefore, the objective of this thesis was to examine the effects of dairy products and non-dairy alternatives consumed with high GI carbohydrate at breakfast on satiety, FI and PPG. Two studies were conducted in which young adults (Experiment 1) were fed dairy and non-dairy alternatives with a breakfast cereal and older adults (Experiment 2) were provided the treatments while consuming bread and jam. The goal of this research was to provide further understanding of the potential functional roles of dairy as an aid in the management of obesity and glycemic control, as well as provide insight into the mechanisms involved.
Chapter 3
Hypotheses and Objectives

3.1 General Hypothesis and Objective

Hypothesis

- Dairy products consumed with high glycemic carbohydrate at breakfast increase satiety and decrease FI and PPG.

Objective

- To identify the effects of dairy products when consumed with high glycemic carbohydrates on subjective appetite, FI and PPG.

3.2 Specific Hypotheses and Objectives

Chapter 4: Experiment 1. THE EFFECT OF DAIRY AND NON-DAIRY BEVERAGES CONSUMED WITH HIGH GLYCEMIC CEREAL ON SATIETY, FOOD INTAKE AND POST-PRANDIAL GLYCEMIA IN YOUNG ADULTS

Hypothesis

- When consumed with high glycemic cereal, dairy beverages increase satiety and decrease FI and PPG more than non-dairy beverages.

Objective

- To investigate the effects of isovolumetric amounts, representing one serving size (250 mL), of 1% milk, a yogurt beverage, a soy beverage, an almond beverage and water consumed with breakfast cereal on subjective appetite, ad libitum FI 120 min later and glycemic response post-treatment and post-meal in healthy young men and women aged 20-30 years old.
Chapter 5: Experiment 2. THE EFFECT OF DAIRY PRODUCTS CONSUMED WITH HIGH GLYCEMIC CARBOHYDRATE ON SATIETY, FOOD INTAKE AND POST-PRANDIAL GLYCEMIA IN OLDER ADULTS

Hypothesis

- When consumed with high glycemic carbohydrate, solid and semi-solid dairy products affect satiety and decrease FI and PPG more than dairy and non-dairy beverages.

Objective

- To investigate the effects of isocaloric breakfasts (380 kcal) containing one serving of 2% milk (250 mL), 2% Greek yogurt (175 g), Cheddar cheese (30 g), a soy beverage (250 mL) with two pieces of bread with jam and water alone (250 mL, 0 kcal meal) on subjective appetite, ad libitum FI 180 min later and glycemic response post-treatment and post-meal in healthy older men and women aged 60-70 years old.
Chapter 4

The effect of dairy and non-dairy beverages consumed with high glycemic cereal on satiety, food intake and post-prandial glycemia in young adults

4.1 Abstract

Objective: The objective was to compare the effect of dairy and non-dairy beverages when consumed with glycemic carbohydrate on satiety, FI and PPG in healthy young adults.

Design: Twenty-six healthy males and females (sex: 13 males and 13 females; age: 23.0 ± 2.6 years; BMI: 22.3 ± 1.5 kg/m²) participated in a randomized crossover study. Treatments were 250 mL of 1% milk, yogurt beverage, soy beverage, almond beverage and water consumed with cereal. At 120 min after consumption, the participants were fed an ad libitum meal. Subjective appetite, blood glucose and insulin were measured at baseline and at intervals both before (post-treatment) and after the meal (post-meal).

Results: Despite higher carbohydrate contents of dairy beverages, blood glucose was similar to non-dairy beverages when consumed with cereal. Soy beverage resulted in the lowest blood glucose post-treatment compared to yogurt beverage, milk and almond beverage (p < 0.0001) but was higher than all in the post-meal (p < 0.0001). Dairy products produced the highest insulin concentrations post-treatment (p < 0.0001). All treatments equally reduced appetite compared to W with cereal (p < 0.0001) but milk resulted in the lowest FI (p < 0.0001).

Conclusion: Protein-containing beverages consumed with a high glycemic cereal at breakfast increase satiety and lower FI and PPG compared to water with cereal.

This study was registered at clinicaltrials.gov as NCT02491814.
4.2 Introduction

With worldwide prevalence doubling since the 1980s, obesity has quickly become a global health crisis [1]. Overweight and obese individuals are most often characterized by other co-morbidities such as T2D for which the risk is increased fourfold [6]. As a result, there is a need to identify dietary measures that can aid in the prevention and/or management of obesity and T2D.

Frequent dairy consumption is associated with lower incidences of obesity and T2D [12, 13]. High-dairy diets are effective interventions for short-term weight loss [14] and for improving T2D risk factors such as insulin resistance [15, 16]. Milk proteins, especially whey, are known to contribute to the regulation of satiety, FI and PPG [18, 45, 48, 49, 59]. However, whole milk has greater effects on regulation than that predicted from the additive effects of its components, highlighting the importance of consuming milk as a whole entity [55, 74]. Dairy beverages are often reported to promote satiety but effects on FI are less clear. Failure to show consistent results for FI across studies can be explained by the variability in study designs regarding energy and macronutrient content of treatments, length of time between treatment and test meal and sample size. For example, both 600 and 240 mL of skim and 1.5% milk led to higher satiety and lower FI than isocaloric amounts of fruit juice [56, 57], but there were no differences in FI when 500 mL of chocolate and skim milk were compared to regular and diet cola [124, 125]. Similarly, drinkable yogurts were more satiating than chocolate bars, crackers and fruit juice but failed to show consistent decreases in FI [77-79]. Furthermore, energy instead of macronutrient content has been shown to be the main determinant of FI following consumption of milk products [94].
Since the development of the GI, there has been a general recommendation to limit the consumption of high GI foods due to their perceived negative effects on blood glucose [128, 129]. However, the PPG response to high GI foods is markedly reduced when they are consumed with a source of protein [130-132]. Milk and milk proteins attenuate PPG when consumed alone [62] or with glycemic carbohydrate [95, 126]. When ad libitum amounts of water, 1% milk, cola, diet cola and orange juice were consumed along with an ad libitum pizza meal, blood glucose was lowest after milk despite no differences in fluid volumes and FI [126]. In another study, white bread consumed with 1% milk or soy beverage resulted in lower blood glucose response than white bread alone [95].

Still, few studies have investigated the effect of dairy beverages or their substitutes when consumed in typical serving sizes and as part of a meal on satiety, FI and PPG. Milk is most commonly consumed at breakfast time and is included in almost half (46%) of breakfast meals [127]. However, breakfast consumption amongst all age groups has declined over the past few decades [127, 133]. As well, the growth in popularity of non-dairy substitutes such as soy and almond beverages [82] has contributed to the decrease in milk consumption. These substitutes are often lower in energy and protein than milk, but their effects on PPG when consumed in a meal as a substitute for milk is unclear.

Therefore, the objective of the present study was to compare the effects of isovolumetric amounts of 1% milk, drinkable yogurt, soy beverage, almond beverage and water consumed with glycemic carbohydrate on subjective appetite, FI 120 min later and blood glucose in healthy young men and women. We hypothesized that dairy beverages consumed with breakfast cereal will increase satiety and decrease FI and PPG more than non-dairy beverages.
4.3 Participants and Methods

4.3.1 Study Design

This experiment was a randomized, non-blinded, crossover design. Men were provided each treatment once per week but for women only during the first two weeks of their menstrual cycle. The order of treatments was randomized using a randomization block design generated with a random generator script in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Treatments were 1% milk, yogurt beverage, soy beverage, almond beverage and water (control). Each treatment was consumed with cereal as part of a breakfast meal. An ad libitum meal was served 120 min following the breakfast meal. Satiety, blood glucose and insulin were measured at baseline and at intervals before and after the ad libitum meal, during which FI was measured.

4.3.2 Participants

Men and women between the ages of 20-30 years old with a body mass index (BMI) of 20.0-24.9 kg/m² were eligible to participate. Participants were recruited through advertisements posted online and around the campus of the University of Toronto. Exclusion criteria included smokers, breakfast skippers, lactose intolerance and allergies to study foods, dislike of study foods, trying to lose or gain weight, elite athletes, use of protein or fibre supplements, prescription medication (excluding hormonal contraceptives), fasting blood glucose of ≥5.6 mmol/L, diabetes or any medical conditions that could interfere with study outcomes. Restrained eaters as identified by a score of ≥ 11 on the Eating Habits Questionnaire were also excluded [134]. For women, regular monthly menstrual cycles were a requirement. Since impaired insulin sensitivity has been observed during the luteal phase of the menstrual cycle [135], females were scheduled only during their follicular phase. The sample size required was based on previous
short-term FI and glycemia studies with dairy [62, 94]. Participants were financially compensated and the study protocol was approved by the Human Subject Review Committee at the University of Toronto Ethics Review Office.

4.3.3 Treatments

Treatments included isovolumetric amounts (250 mL) of: 1) 1% milk (Neilson Dairy; ON, Canada), 2) yogurt beverage (Yop Vanilla; General Mills; ON, Canada), 3) soy beverage (Original Organic; Silk; CO, USA), 4) almond beverage (Original; Silk; CO, USA) and 5) water (control). Each treatment was part of a breakfast meal that also included 54 g of Cheerios cereal (General Mills; ON, Canada). The nutritional composition of each treatment and the cereal is outlined in Table 4.1.

All treatments were served chilled in a cup. Participants were instructed to pour the treatment into the bowl of cereal and consume the breakfast with a spoon. They were required to finish any leftover liquid in the bowl. An additional 100 mL of water was provided to reduce the aftertaste of the breakfast meal which participants were instructed to drink only when they had finished eating.

4.3.4 Protocol

Each participant was scheduled to arrive at the same time (between 8:30 and 10 am) and day of the week to the Department of Nutritional Sciences at the University of Toronto for each of their five study sessions. Participants were required to observe a 12 h overnight fast, except for water, which was permitted until 1 h before the session. They were also instructed to refrain from vigorous exercise and alcohol consumption the day before their sessions and to eat similarly the evenings before.
Upon arrival, participants completed questionnaires to ensure no unusual deviations from their diet and lifestyle patterns from the previous day and current morning. If they were deemed unfit to participate (e.g. due to sickness), they were rescheduled. Participants then completed visual analogue scale (VAS) questionnaires to assess their “Food Intake and Activity Level”, “Sleep Habits”, “Stress Factors”, “Feelings of Fatigue”, “Physical Comfort” and “Motivation to Eat” [62, 94]. The “Motivation to Eat” VAS consisted of four appetite questions assessing the participants’ “desire to eat”, “hunger”, “fullness” and “prospective food consumption”. The scores from these four questions were averaged to obtain the overall average appetite score for statistical analysis [136-138]. The score for “fullness” was subtracted from 100 before inclusion into the calculation.

Participants provided a baseline finger prick capillary blood sample using a single-use lancet (Unistik; Oxfordshire, UK). Plasma blood glucose concentration was measured with a glucometer (Accu-Chek Aviva; Roche Diagnostics Canada, Laval, QB, Canada). A baseline measurement of >5.6 mmol/L suggested non-compliance with the fasting instructions or high fasting plasma glucose and the participant was rescheduled or excluded from the study.

Participants were instructed to consume the breakfast meal within 10 min while eating at a steady pace. After consumption, the palatability of the treatment was measured by VAS. Subjective appetite and blood glucose were measured at 15, 30, 45, 60, 75, 90 and 120 min (post-treatment period) from the time participants began eating the breakfast meal and insulin was measured at 0 (baseline), 30, 60, 90 and 120 min. Blood for insulin analysis was collected via finger prick (as previous described) with Microvette capillary tubes 300 Z (Sarstedt, Numbrecht, Germany). Blood was then immediately centrifuged (20°C, 10000 rpm, 5 min) and the plasma were frozen at -80°C until measurements. Insulin levels were quantified with a commercially
available Insulin enzyme immunoassay kit (Alpco, Salem, New Hampshire). Participants were asked to remain seated throughout the session and were permitted to do quiet activities such as read or listen to music.

At 120 min, participants were fed an ad libitum test meal. Participants were provided with three varieties of pizza (Deluxe, Pepperoni and Three Cheese; McCain Foods Ltd., Florenceville, NB) based on their preferences at the initial screening. They were allowed 20 min to eat and instructed to eat until they felt “comfortably full”. Three separate trays of pizza were provided at regular intervals regardless if the participant was finished eating the current tray or already full. Subjective appetite, blood glucose and insulin were measured at 140 and 170 min (post-meal period).

Detailed information regarding the nutrient content of the pizzas and method of cooking was reported previously [139]. Test meal consumption was calculated by weighing the amount of pizza consumed and based on the nutritional information provided by the manufacturer. Ad libitum water intake at the test meal was measured by weight (g).

Cumulative energy intake was calculated by adding the caloric content of the fixed breakfast with the calories consumed at the test pizza meal. Caloric compensation, expressed as a percentage, was calculated by subtracting the calories consumed at the test meal following the treatment from those following the water control divided by the calories in the treatment and multiplied by 100. A caloric compensation of <100% indicates undercompensation meaning that the energy consumed at the test meal plus the energy of the treatment (beverage only) exceeded the energy consumed at the test meal following the control (water), while the opposite is known as overcompensation and is indicated by a score >100% [94].
4.3.5 Statistical Analyses

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). Two and three-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of treatment, time, sex and their interaction on dependent variables over the entire study period including changes from baseline for average appetite scores, blood glucose and insulin concentrations and means for FI, cumulative energy intake, water intake and caloric compensation. There were no significant interactions between treatment and sex for any of the variables therefore these results were pooled for both men and women. When there was a significant treatment-by-time interaction, one-factor ANOVA (PROC MIXED procedure) was followed by Tukey’s post-hoc test to investigate the effect of treatment on changes from baseline for appetite, blood glucose and insulin at each time of measurement and means for FI, cumulative energy intake, water intake and caloric compensation. Post-treatment changes from baseline were calculated from 15-120 min and post-meal changes from 140-170 min.

Incremental areas under the curve (iAUC) were calculated for blood glucose and insulin separately for the post-treatment and the post-meal period; post-treatment and post-meal periods were 0-120 min and 120-170 min respectively for all variables. Glucose to insulin ratios were compared among treatments by calculating both the ratios of blood glucose to insulin iAUCs (mmol min L\(^{-1}/\mu U\) mL\(^{-1}\)) post-treatment and post-meal, and the ratios of changes from baseline of blood glucose to insulin at 30 min (mmol min L\(^{-1}/\mu U\) mL\(^{-1}\)). Glucose to insulin ratio measures the efficacy of insulin action [140]. The lower the ratio is, the higher the efficacy of insulin action. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was set at \(p < 0.05\).
4.4 Results

4.4.1 Participant Characteristics

Twenty-six healthy males and females (sex: 13 males and 13 females; age: 23.0 ± 2.6 years; BMI: 22.3 ± 1.5 kg/m²) completed all five sessions. Dropouts include one female participant who was excluded due to failure to schedule subsequent sessions and two males due to concurrent participation in another intervention study. Their results were not included in the analysis.

4.4.2 Palatability of treatments

Treatment had an effect on palatability scores (p < 0.0001). Water with cereal was the least preferred compared to all other treatments but there were no differences between other treatments.

4.4.3 Food and Water Intake

There was an effect of treatment on FI (p < 0.0001), cumulative energy intake (p < 0.0001) and caloric compensation (p = 0.0045) but not on water intake (Table 4.2). All treatments resulted in lower FI at the ad libitum meal than water. Energy intake was lowest after milk but was not different than yogurt beverage. Cumulative energy intake (breakfast + lunch meal) was also lowest after milk but milk was not different from almond beverage. Similarly, compensation for energy in the beverages was greater for milk compared to soy and yogurt beverages but similar to that for almond beverage. Only milk and almond beverage led to overcompensation (> 100%) at the ad libitum meal.
4.4.4 Subjective Appetite

Post-treatment (15-120 min) mean appetite changes from baseline were affected by time ($p < 0.0001$) and treatment ($p < 0.0001$) but not by a time-by-treatment interaction. A greater decrease in appetite, expressed as changes from baseline, occurred following yogurt beverage compared to water at 15, 30, 60, 75, 90 and 120 min. At 15 min, almond beverage also led to lower appetite than water (Fig. 4.1). Post-meal (140-170 min) mean appetite changes from baseline were only affected by time ($p < 0.0001$). There were no differences between any of the treatments at 140 or 170 min (Fig. 4.1).

Over the entire post-treatment period (15-120 min), yogurt beverage suppressed appetite compared to all treatments ($p < 0.0001$; Table 4.3). Almond beverage suppressed appetite compared to water and soy beverage. There were no differences between treatments in the post-meal period (140-170 min) (Table 4.3). Cumulative (15-170 min) appetite was similar to the post-treatment period except almond beverage also suppressed appetite compared to milk ($p < 0.0001$; Table 4.3).

4.4.5 Blood Glucose Concentrations

Post-treatment (15-120 min) blood glucose mean changes from baseline were affected by treatment ($p < 0.0001$), time ($p < 0.0001$) and by a time-by-treatment interaction ($p < 0.0001$). Blood glucose, expressed as changes from baseline, peaked at 30 min and failed to return to baseline for all treatments. At 15 min, blood glucose was lowest for soy beverage compared to all treatments except milk. Blood glucose at 30 min was still lowest after soy beverage but was no longer different from almond beverage. At 45 min, all treatments attenuated blood glucose compared to water but by 60 min, blood glucose was only lower for soy beverage and milk. By
90 and 120 min, blood glucose was highest for almond beverage compared to water with no differences between any other treatments (Fig. 4.2). Post-meal (140-170 min) blood glucose mean changes from baseline were affected by treatment \((p < 0.0001)\), time \((p < 0.0001)\) and by a time-by-treatment interaction \((p = 0.001)\). Blood glucose was highest for water and soy beverage compared to yogurt beverage at 170 min. There were no differences between any other treatments (Fig. 4.2).

Over the entire post-treatment period (15-120 min), blood glucose was lowest for soy beverage compared to all other treatments \((p < 0.0001; \text{Table } 4.3)\). In addition, milk led to lower blood glucose than water \((p < 0.0001)\) but was not different from yogurt beverage and almond beverage (Table 4.3). Over the entire post-meal period (140-170 min), blood glucose was lowest following almond beverage and yogurt beverage and highest following water \((p < 0.0001; \text{Table } 4.3)\).

Cumulative (15-170 min) blood glucose for all treatments was lower than water except almond beverage with soy beverage resulting in the lowest blood glucose compared to all treatments except milk \((p < 0.0001; \text{Table } 4.3)\).

4.4.6 Insulin Concentrations

Post-treatment (15-120 min) mean insulin changes from baseline were affected by treatment \((p<0.0001)\), time \((p<0.0001)\) and by a time-by-treatment interaction \((p<0.0001)\). Insulin, expressed as changes from baseline, peaked at 30 min for all treatments and failed to return to baseline within the post-treatment period. At 30 and 60 min, insulin was highest following yogurt beverage compared to all treatments. Additionally, milk led to higher insulin than soy beverage and almond beverage which were not different from each other. At 90 min, insulin was highest for yogurt beverage which was not different from milk and almond beverage and lowest after water which was not different from soy beverage. Insulin at 120 min was the same as 90
min, except that almond beverage was no longer the same as yogurt beverage (Fig. 4.3). Post-meal (140-170 min) mean insulin changes from baseline were only affected by time \((p<0.0001)\) and by a time-by-treatment interaction \((p<0.0001)\). Insulin was highest for yogurt beverage compared to water at 140 min with no differences between any other treatments. There were no differences between treatments at 170 min (Fig. 4.3).

Over the entire post-treatment period (15-120 min), insulin was highest for yogurt beverage compared to all other treatments \((p < 0.0001)\). Milk resulted in higher insulin than water, soy beverage and almond beverage with no differences between those treatments \((p < 0.0001; \text{Table 4.3})\). There were no differences between treatments in the post-meal (140-170 min) period (Table 4.3). Cumulative (0-170 min) insulin was similar to the post-treatment period except that milk was not different from water and soy beverage \((p < 0.0001; \text{Table 4.3})\).

4.4.7 Blood Glucose to Insulin Ratios

The ratios of blood glucose to insulin iAUCs were calculated for the post-treatment (0-120 min) and post-meal (120-170 min) periods. In the post-treatment period, yogurt beverage led to the lowest ratio but was not different from milk and soy beverage \((p < 0.0001; \text{Table 4.4})\). Almond beverage led to the highest ratio but was not different from water \((p < 0.0001)\). There were no differences between any treatments post-meal (Table 4.4). Cumulative (0-170 min) results were the same as the post-treatment. When the ratios for mean changes from baseline at 30 min were considered, it was lowest following yogurt beverage and highest following water \((p = 0.03)\) with no differences between any other treatments (Table 4.4).
4.5 Discussion

In contrast to our hypothesis, all dairy and non-dairy beverages consumed with a glycemic breakfast cereal resulted in similarly reduced appetite, PPG as well as FI at a later meal. The peak increase in blood glucose from 30-60 min was 30% lower when cereal was consumed with these beverages than with water. Thus, the results show that co-ingestion of carbohydrates with dairy and non-dairy beverages increase satiety and decrease FI and PPG compared to carbohydrates alone.

The glycemic responses of the treatments were not determined by their carbohydrate contents. Despite having 27 and 41% more carbohydrate than the entire almond beverage and water breakfast meals, respectively, yogurt beverage led to the same glycemic response in the post-treatment period (0-120 min). This is similar with a study that showed reduced blood glucose after chocolate milk compared to orange juice despite higher sugar content [94]. When only the first hour (0-60 min) is taken into consideration, yogurt beverage, almond beverage and milk are all significantly lower than water with no differences between them ($p < 0.0001$; data not shown). This indicates that the initial blood glucose response of yogurt beverage, almond beverage and milk are much lower than that of water which is important when considering the physiological relevance of using these beverages as dietary interventions for glycemic control.

The attenuation in blood glucose may be attributed to the milk proteins in yogurt beverage since it is well-known that carbohydrate consumed with protein leads to lower blood glucose than carbohydrate alone. This is further supported by the low glucose response of soy beverage which only had 2 g less carbohydrate than almond beverage but 6 g more protein. However, although milk had 3 g more protein than yogurt beverage, they resulted in the same blood glucose
response indicating that protein was not the only factor contributing to the attenuation of blood glucose by yogurt beverage. Milk proteins stimulate insulin [42] but non-insulin dependent mechanisms also determine insulin and blood glucose responses. Fermentation is a possible explanation since fermented milk beverages delay gastric emptying when compared to regular milks [73, 141] which contributes to attenuation of blood glucose. Delayed gastric emptying by yogurt beverage is suggested by the low blood glucose response at 170 min (30 min after test meal) (Fig. 4.2). The higher energy [105] and fat content [69] of yogurt beverage may have also contributed to this effect.

An unexpected finding was the delayed fall in blood glucose possibly due to a delay in gastric emptying following almond beverage. When only looking at the second hour (75-120 min), blood glucose is significantly higher for almond beverage compared to all other treatments ($p = 0.0006$; data not shown). This indicates a sustained blood glucose response throughout the post-treatment period that carried over to the post-meal period as evidenced by the low glucose response at 170 min (30 min after test meal). Commercially-available non-dairy beverages often contain additives such as thickening agents. Locust bean gum, sunflower lecithin and gellan gum were ingredients in the brand of almond beverage used. Locust bean gum has been reported to delay gastric emptying when added to rice puddings [142] and infant formula [143] and gellan gum delayed gastric emptying in rats [144]. Delay in gastric emptying may also explain the lower, although non-significant, glucose response relative to milk in the post-meal despite higher FI. Similarly, the brand of soy beverage used contained carrageenan which may provide an explanation for the attenuation of blood glucose by soy beverage compared to milk. When 2.5 g of carrageenan was added to 250 mL of 3.25% milk, blood glucose was significantly lower than 3.25% milk alone at 30 min which is the same as the results for soy beverage and milk in the
present study [145]. The blood glucose response curve of milk with added carrageenan in that study, which is similar to the response curve of soy beverage in the present study, shows a sustained blood glucose response which the authors explain by delayed gastric emptying. However, it is unclear what type and how much carrageenan is in this soy beverage.

Insulin responses corresponded with both the carbohydrate and protein content although the differences in macronutrient content of the treatments make it difficult to distinguish the degree of effect for each macronutrient. At the peak time of 30 min, yogurt beverage led to 38% more insulin than water despite producing 3% lower glucose response and milk led to 20% more insulin than water despite producing 12% lower glucose response. Therefore, there is evidence of non-glucose-dependent insulin release and supports the idea of protein-stimulated insulin release since milk proteins are known to be insulinotropic [42]. This is further demonstrated by soy protein, which has also been shown to be insulinotropic [146], since soy beverage led to 21% more insulin despite leading to 24% lower glucose compared to almond beverage. Throughout the post-treatment period, insulin for yogurt beverage remained higher at all measured times even when its blood glucose response was similar or lower (non-significant) than non-dairy beverages. This sustained insulin response is likely due to an additive effect of the insulinotropic milk proteins and carbohydrate content since milk had more protein but did not result in higher insulin than yogurt beverage. Sun et al. showed that when soy beverage (10.8 g sucrose, 8.6 g protein) consumed with white bread was compared to 1% milk (15.1 g lactose, 8.6 g protein) with bread, insulin was higher following soy beverage [95]. This indicates that lower insulin response by soy beverage compared to milk in the present study may be due to a combination of the higher protein content of milk and the delay in gastric emptying by soy beverage. However, blood glucose to insulin iAUC ratio in the post-treatment period were similar amongst protein-
containing treatments (milk, yogurt beverage and soy beverage) and lower than the treatments without protein (Table 4.3). Therefore, enhanced efficacy of insulin activity mediated by proteins likely played a role in improving reducing PPG.

Satiety in the post-treatment period was not determined by protein content. Yogurt led to the lowest subjective appetite ratings which is likely due to its high energy content [94]. Additionally, sweetness has been shown to reduce appetite [147] and the yogurt beverage contained a large amount of added sugar and was the sweetest tasting treatment. However, subjective appetite ratings were not predictive of FI which is consistent with previous studies [79, 124].

Milk resulted in lower FI compared to non-dairy beverages. This is contrary to a previous study that compared soy beverage and milk when consumed alone [94]. In that study, there were no differences in FI measured at 120 min indicating perhaps a stronger effect on FI when milk is consumed with a meal. In addition, soy protein is considered a “fast” protein and milk is primarily made up of casein, a “slow” protein that coagulates in the stomach and delays gastric emptying [93]. Therefore, lower FI may be attributed to the actions of casein. It is unlikely that energy content of the treatments played a role in affecting FI since the difference between milk and soy beverage was only 10 calories and yogurt beverage resulted in the same FI as soy beverage and almond beverage despite much higher calories. Furthermore, lower FI for milk translated to lower cumulative FI throughout the entire study session which is supported by overcompensation (190%) at the test meal. This indicates that milk is a good choice of beverage for the purpose of reducing energy intake at a later meal although longer-term effects need to be studied. Overcompensation (167%) by almond beverage was likely due to its low energy content (60 kcal) while undercompensation (71%) by yogurt beverage was likely due to its high energy content.
content (188 kcal) contributed mostly from the added sugars in the product. Therefore, an unsweetened yogurt beverage may have produced different results on blood glucose, insulin and caloric compensation however none were available on the market.

The lack of control for the macronutrient energy content of treatments may be seen as a limitation of this study. However, the objective was to compare commercially-available products consumed in typical serving sizes at usual meals and therefore provide data with ecological validity. A limitation of this study design was the ad libitum nature of the test meal which does not allow for optimal measurement of a second-meal effect for glycemia and potentially causes blunting of treatment effects on insulin in the post-meal period. A fixed-meal based on calories per kilogram bodyweight such as one described in a previous study by Akhavan et al. [18] would have been more effective however the measurement of FI requires ad libitum feeding. Furthermore, the measurement of gastrointestinal hormones as well as gastric emptying through methods like paracetamol administration would have contributed to better understanding of the mechanisms involved in appetite and glycemic regulation by dairy and non-dairy beverages. Finally, although the study included both men and women, the results cannot be extrapolated to unhealthy populations such as obese, pre-diabetic and diabetic individuals as well as the elderly which are all groups that would greatly benefit from dietary measures of appetite and glycemic control.

In summary, all dairy and non-dairy beverages tested in this study led to increased satiety and reduced FI and PPG when consumed with a glycemic cereal versus cereal alone. Consistent with previous studies [94, 95], blood glucose and insulin responses were not predicted only by carbohydrate content. The marked effect on reducing glucose responses to the cereal suggests that dietary advice based on the avoidance of high GI carbohydrates should not be taken at face
value as has also been previously stated [130]. A greater appreciation of the complementary benefits of food and food components is needed.

4.6 Conclusion

Protein-containing beverages consumed with a high glycemic cereal at breakfast increase satiety and lower FI and PPG compared to water with cereal.
### Table 4.1
Nutritional information of treatments and breakfast cereal

<table>
<thead>
<tr>
<th>Nutrients B</th>
<th>Soy beverage</th>
<th>Almond beverage</th>
<th>1% Milk</th>
<th>Yogurt beverage</th>
<th>Cereal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>100</td>
<td>60</td>
<td>110</td>
<td>188</td>
<td>200</td>
</tr>
<tr>
<td>Fat (total) (g)</td>
<td>4</td>
<td>3</td>
<td>2.5</td>
<td>5.6</td>
<td>3</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>0.5</td>
<td>0.2</td>
<td>1.5</td>
<td>3.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Trans fat (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>110</td>
<td>160</td>
<td>120</td>
<td>68.8</td>
<td>340</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>8</td>
<td>9</td>
<td>12</td>
<td>27.5</td>
<td>40</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>5</td>
<td>8</td>
<td>12</td>
<td>27.5</td>
<td>2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>6.3</td>
<td>6</td>
</tr>
</tbody>
</table>

A Treatments consumed with cereal.

B Nutrient content of each product is provided by the manufacturer. Amounts given are per 250 mL of each treatment and 54 g of cereal.
Table 4.2
Energy intake, cumulative energy intake, water intake and caloric compensation.\(^A\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Energy intake (kcal)</th>
<th>Water Intake (g)</th>
<th>Caloric Compensation (%)(^D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test meal(^B)</td>
<td>Cumulative(^C)</td>
<td></td>
</tr>
<tr>
<td>Control(^E)</td>
<td>835.8±66.8(^a)</td>
<td>1035.8±66.8(^{ab})</td>
<td>322.9±30.5</td>
</tr>
<tr>
<td>Soy beverage</td>
<td>743.6±57.5(^b)</td>
<td>1043.6±57.5(^{ab})</td>
<td>336.5±29.8</td>
</tr>
<tr>
<td>Almond beverage</td>
<td>726.2±52.4(^b)</td>
<td>986.2±52.4(^{bc})</td>
<td>311.6±30.2</td>
</tr>
<tr>
<td>1% Milk</td>
<td>624.3±41.3(^c)</td>
<td>934.3±41.3(^c)</td>
<td>328.4±27.8</td>
</tr>
<tr>
<td>Yogurt beverage</td>
<td>700.2±47.1(^{bc})</td>
<td>1088.2±47.1(^a)</td>
<td>358.0±26.3</td>
</tr>
</tbody>
</table>

\(^p\) <0.0001    \(<0.0001\)    NS    0.0045

\(^A\) All values are means ± SEMs (n = 26). Values in the same column with different superscript letters are significantly different. \(P < 0.05\) (treatment effect using proc mixed, Tukey’s post-hoc).

\(^B\) Energy consumed in an ad libitum meal was measured at 120 min following treatment consumption.

\(^C\) Energy in breakfast meal (treatment + cereal) + energy from meal.

\(^D\) Caloric compensation = [(kcal consumed at meal after the water control – kcal consumed at the meal after the beverage)/kcal in the beverage] x 100.

\(^E\) Water (250 mL).
Table 4.3
Effect of treatment on post-treatment, post-meal and cumulative mean changes from baseline for subjective appetite, blood glucose and insulin

<table>
<thead>
<tr>
<th></th>
<th>Control(^B)</th>
<th>Soy beverage</th>
<th>Almond beverage</th>
<th>1% Milk</th>
<th>Yogurt beverage</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>-23.6±1.8(^a)</td>
<td>-26.2±1.8(^a)</td>
<td>-29.7±1.7(^b)</td>
<td>-26.5±1.7(^ab)</td>
<td>-35.3±1.8(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-22.6±1.8</td>
<td>-26.2±1.8</td>
<td>-29.7±1.7</td>
<td>-26.5±1.7</td>
<td>-35.4±1.8</td>
<td>NS(^F)</td>
</tr>
<tr>
<td>Cumulative</td>
<td>-31.3±1.8(^a)</td>
<td>-32.7±1.8(^a)</td>
<td>-35.8±1.7(^b)</td>
<td>-32.3±1.7(^ab)</td>
<td>-40.6±1.7(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>1.0±0.08(^a)</td>
<td>0.7±0.05(^c)</td>
<td>0.9±0.05(^ab)</td>
<td>0.9±0.06(^b)</td>
<td>0.9±0.06(^ab)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-meal</td>
<td>0.8±0.1(^a)</td>
<td>0.6±0.09(^ab)</td>
<td>0.3±0.06(^c)</td>
<td>0.5±0.09(^bc)</td>
<td>0.4±0.06(^bc)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cumulative</td>
<td>1.2±0.07(^a)</td>
<td>0.9±0.05(^c)</td>
<td>1.1±0.05(^ab)</td>
<td>1.0±0.05(^bc)</td>
<td>1.0±0.06(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>18.2±2.3(^c)</td>
<td>18.7±1.9(^c)</td>
<td>16.5±1.6(^c)</td>
<td>25.7±2.6(^b)</td>
<td>33.9±3.2(^a)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-meal</td>
<td>38.2±4.2</td>
<td>39.7±4.5</td>
<td>36.5±3.2</td>
<td>36.1±2.9</td>
<td>43.7±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative</td>
<td>28.8±2.4(^bc)</td>
<td>29.1±2.4(^bc)</td>
<td>26.0±1.9(^c)</td>
<td>32.1±2.2(^b)</td>
<td>40.4±2.7(^a)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^A\) All values are means ± SEMs (\(n = 26\)). Means within a row with different superscript letters are significantly different, \(P < 0.05\) (treatment using proc mixed, Tukey’s post hoc).

\(^B\) Water (250 mL)

\(^C\) Post-treatment values are mean changes from baseline of all observations before the test meal: 15, 30, 45, 60, 75, 90 and 120 min.

\(^D\) Post-meal values are mean changes from baseline of all observations after the test meal: 140 and 170 min.

\(^E\) Cumulative values are mean changes from baseline for all observations for the entire session: 15-170 min.

\(^F\) NS = non significant.
**Table 4.4**

Effect of treatment on post-treatment, post-meal and cumulative ratios of blood glucose to insulin iAUC and on ratios of blood glucose to insulin changes from baseline at 30 min

<table>
<thead>
<tr>
<th></th>
<th>Control B</th>
<th>Soy beverage</th>
<th>Almond beverage</th>
<th>1% Milk</th>
<th>Yogurt beverage</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose/insulin iAUC ratio (mmol min L⁻¹/µU min mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment C</td>
<td>0.06±0.007ab</td>
<td>0.04±0.004bc</td>
<td>0.07±0.01a</td>
<td>0.04±0.006bc</td>
<td>0.03±0.004c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-meal D</td>
<td>0.03±0.004</td>
<td>0.02±0.003</td>
<td>0.02±0.002</td>
<td>0.02±0.003</td>
<td>0.02±0.004</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative E</td>
<td>0.04±0.003a</td>
<td>0.04±0.004ab</td>
<td>0.05±0.005a</td>
<td>0.04±0.005ab</td>
<td>0.03±0.003b</td>
<td>0.0006</td>
</tr>
<tr>
<td>Glucose/insulin changes from baseline ratio (mmol min L⁻¹/µU min mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>0.20±0.04a</td>
<td>0.16±0.03ab</td>
<td>0.18±0.02ab</td>
<td>0.14±0.03ab</td>
<td>0.09±0.008b</td>
<td>0.0346</td>
</tr>
</tbody>
</table>

A All values are means ± SEMs (n = 30). Means within a row with different superscript letters are significantly different, P < 0.05 (treatment using proc mixed, Tukey’s post hoc).
B Water (250 mL)
C Post-treatment values are mean changes from baseline of all observations before the test meal: 0, 15, 30, 45, 60, 75, 90 and 120 min.
D Post-meal values are mean changes from baseline of all observations after the test meal: 120, 140 and 170 min.
E Cumulative values are mean changes from baseline for all observations for the entire session: 0-170 min.
F NS = non significant.
Fig. 4.1. Effect of treatments on average subjective appetite mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 26$).

Fig. 4.2. Effect of treatments on blood glucose mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 26$).
Fig. 3. Effect of treatments on insulin mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 26$).
Chapter 5

The effect of dairy products consumed with high glycemic carbohydrate on satiety, food intake and post-prandial glycemia in older adults

5.1 Abstract

**Objective:** The objective was to compare the effect of liquid, semi-solid and solid dairy products and a non-dairy beverage when consumed with glycemic carbohydrate on satiety, FI and PPG in healthy older adults.

**Design:** Thirty healthy males and females (sex: 14 males and 16 females; age: 64.6 ± 2.4 y; BMI: 25.6 ± 2.5 kg/m²) participated in a randomized crossover study. Treatments were 250 mL of 2% milk and soy beverage, 175 g of 2% Greek yogurt and 30 g of Cheddar cheese consumed with bread and jam. At 180 min after consumption, the participants were fed an ad libitum meal. Subjective appetite, blood glucose and insulin were measured at baseline and at intervals both before (post-treatment) and after the meal (post-meal).

**Results:** Cheese and yogurt resulted in lower post-treatment blood glucose than milk and soy beverage ($p < 0.0001$) but no differences between treatments were observed post-meal. All treatments led to similar insulin concentrations. Post-treatment appetite was suppressed by cheese and yogurt compared to milk ($p < 0.0001$) but there were no differences in FI between treatments.

**Conclusions:** Cheese and yogurt consumed with carbohydrate increase satiety and lower PPG more than milk or a soy beverage with carbohydrate and may be effective dietary interventions.
for appetite and glycemic control in older adults.

This study was registered at clinicaltrials.gov as NCT02607007.

5.2 Introduction

Foods that increase satiety and decrease PPG may be effective dietary interventions for the prevention and management of obesity and T2D [148, 149]. To encourage development and identification of such foods, government regulatory agencies have released guidance documents for food health claims related to appetite and blood glucose control. In 2012, the European Food Safety Authority (EFSA) released their document titled “Guidance on the scientific requirements for health claims related to appetite ratings, weight management, and blood glucose concentrations” [9]. Additionally, Health Canada (HC) released two draft guidance documents on food health claims for “satiety” and “the reduction in post-prandial glycemic response” in 2012 and 2013, respectively [10, 11].

Earlier experiments have reported on the effectiveness of milk proteins, especially whey, to increase satiety and reduce FI and PPG. More recently, the focus has shifted to milk as a whole entity. Milk consumed in typical serving sizes (e.g. 240-600 mL) decreases appetite, FI and PPG more when compared to other beverages such as fruit juice and colas [56, 57, 94, 125, 126]. Similar results have also been seen with liquid and semi-solid yogurts [62, 77-79]. Therefore, dairy products are candidates for food health claims related to appetite and glucose control. However, dairy products vary not only in macronutrient composition but also in food form (i.e. liquid, semi-solid and solid) which may be expected to result in different effects on appetite and blood glucose.
Previous studies have shown that solid food forms are more satiating than liquid forms but effects on PPG have not been consistent [150, 151]. However, there has only been one report comparing different dairy forms on appetite and PPG. Dougkas et al. compared isocaloric (201 kcal) amounts of yogurt (278 g), Cheddar cheese (49 g) and semi-skimmed milk (410 mL) containing 10.9 g, 12.3 g and 13.9 g of protein, respectively, and showed that yogurt suppressed hunger the most but there were no differences in FI 90 min later or in blood glucose and insulin, which were only measured at 80 min [81]. When skim milk (16.6 g protein) was compared to three yogurts containing 23.1 g, 22.2 g and 18.3 g of protein, there were no differences in satiety or FI 120 min later or blood glucose before and after the meal [62]. However, the effect of dairy when consumed with meals, such as breakfast containing primarily carbohydrate, has received little attention even though consumption of milk with carbohydrate lowers the blood glucose response compared to carbohydrate alone [95, 126].

Adults post 50 years old are the most at risk group for overweight and obesity and developing T2D due to metabolic inflexibility [22] and inclusion of dairy in their diets has potential to improve and regulate their metabolic outcomes. However, dairy consumption decreases with age [152, 153]. In 2004, only 26 and 20% of Canadian men and women aged 51 to 70 years consumed the recommended amount [21]. In addition, the past few decades non-dairy alternatives have been growing in popularity, competing with milk in the diet and contributing to the decline in general milk consumption [20, 83]. The effects of these beverages in comparison with dairy when consumed with carbohydrate as usually consumed by this age group have not been reported.

Therefore, the objective of the present study was to compare the effects of one serving of 2% milk, 2% Greek yogurt, Cheddar cheese and soy beverage consumed with glycemic carbohydrate
on subjective appetite, FI 180 min later and blood glucose in healthy older men and women. We hypothesized that semi-solid and solid dairy products increase satiety and decrease FI and PPG more than the liquid dairy product and that all dairy products will be more effective than the non-dairy beverage.

5.3 Participants and Methods

5.3.1 Study Design

This experiment was a randomized, non-blinded, crossover design. Participants were provided each treatment once per week in random order using a randomized block design generated with a random generator script in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Treatments were 2% milk, soy beverage, Cheddar cheese, 2% Greek yogurt and water (control). Each treatment was consumed with bread and jam as part of a breakfast meal except for water. An ad libitum meal was served 180 min following the breakfast meal. Satiety, blood glucose and insulin were measured at baseline and at intervals before and after the ad libitum meal, during which FI was measured.

5.3.2 Participants

Men and women between the ages of 60-70 years old with a body mass index (BMI) of 18.5-29.9 kg/m² were eligible to participate. Participants were recruited through advertisements posted online, around the city of Toronto and newspapers. Exclusion criteria included smokers, breakfast skippers, lactose intolerance and allergies to study foods, dislike of study foods, trying to lose or gain weight, fasting blood glucose of > 6.0 mmol/L, diabetes or any prescription medication or medical conditions that could interfere with study outcomes. Restrained eaters as identified by a score of ≥11 on the Eating Habits Questionnaire were also excluded [134].
Women that had not undergone menopause for at least 1 year and who were on hormonal treatments were also excluded. The sample size required was based on previous short-term FI and glycemia studies with dairy [62, 94]. Participants were financially compensated and the study protocol was approved by the Human Subject Review Committee at the University of Toronto Ethics Review Office.

5.3.3 Treatments

Treatments included 1) 2% milk (Neilson Dairy; ON, Canada), 2) soy beverage (Vanilla So Good; BC, Canada); Cheddar cheese (Armstrong; QC, Canada); 2% Greek yogurt (Danone; QC, Canada) and 5) water (control). To equate the volumes of the liquid treatments, water was provided with yogurt and cheese. Each treatment was part of an isocaloric (380 kcal) breakfast meal that also included two pieces (75 g) of white bread (Wonder Bread, ON, Canada) and strawberry jam (Smuckers; ON, Canada). Water alone was served as the control, as recommended by HC for satiety claims [10]. The breakfast meals were made isocaloric by modifying the amount of jam served with each treatment. The serving size and nutritional composition of each treatment and breakfast food is outlined in Table 5.1.

All treatments were served chilled and the bread was provided toasted and served with the jam uniformly spread. An additional 100 mL of water was provided to reduce the aftertaste of the breakfast meal which participants were instructed to drink only when they had finished all the other food and beverages.

5.3.4 Protocol

Each participant was scheduled to arrive at the same time (between 8:30 and 10 am) and day of the week to the Department of Nutritional Sciences at the University of Toronto for each of their
five study sessions. Participants were required to observe a 12 h overnight fast, except for water, which was permitted until 1 h before the session. They were also instructed to refrain from vigorous exercise and alcohol consumption the day before their sessions and to eat similarly the evenings before.

Upon arrival, participants completed questionnaires, as described previously [139], to ensure no unusual deviations from their diet and lifestyle patterns from the previous day and current morning. If they were deemed unfit to participate (e.g. due to sickness), they were rescheduled. Participants then completed VAS questionnaires to assess their “Food Intake and Activity Level”, “Sleep Habits”, “Stress Factors”, “Feelings of Fatigue”, “Physical Comfort” and “Motivation to Eat” [62, 94]. The “Motivation to Eat” VAS consisted of four appetite questions assessing the participants’ “desire to eat”, “hunger”, “fullness” and “prospective food consumption”. The scores from these four questions were averaged to obtain the overall average appetite score for statistical analysis [136-138]. The score for “fullness” was subtracted from 100 before inclusion into the calculation.

Participants provided a baseline finger prick capillary blood sample using a single-use lancet (Unistik; Oxfordshire, UK). Plasma blood glucose concentration was measured with a glucometer (Accu-Chek Aviva; Roche Diagnostics Canada, Laval, QB, Canada). A baseline measurement of $\geq 6.0$ mmol/L suggested non-compliance with the fasting instructions or high fasting blood glucose and the participant was rescheduled or excluded from the study.

Participants were instructed to consume the breakfast meal within 10 min while eating at a steady pace. After consumption, the palatability of the treatment was measured by VAS [138]. Subjective appetite and blood glucose were measured at 15, 30, 45, 60, 90, 120, 150 and 180 min
(post-treatment) from the time participants began eating the breakfast meal and insulin was measured at 0 (baseline), 30, 60, 90, 120, 150 and 180 min. Blood for insulin analysis was collected via finger prick (as previously described) with Microvette capillary tubes 300 Z (Sarstedt, Numbrecht, Germany). Blood was then immediately centrifuged (20°C, 10000 rpm, 5 min) and the plasma were frozen at -80°C until measurements. Insulin levels were quantified with a commercially available Insulin enzyme immunoassay kit (Alpco, Salem, United States).

Participants were asked to remain seated throughout the session and were permitted to do quiet activities such as read or listen to music.

At 180 min, participants were fed an ad libitum test meal. The meal consisted of rice (Uncle Ben’s, ON, Canada), beef meatballs (President’s Choice, ON, Canada) and tomato sauce (Ragu, IL, USA). The meatballs were cut into small and uniform pieces and were mixed homogeneously with the other ingredients in a bowl. One bowl was a 479.5 g portion which represented 827.5 kcal, 30.8 g fat, 104.8 g carbohydrate and 30.2 g protein. Participants were allowed 20 min to eat and were instructed to eat until they felt “comfortably full”. Halfway through the meal (10 min), their bowl was replaced with a new bowl of food regardless if they were finished eating. Subjective appetite, blood glucose and insulin were measured at 210 min (post-meal).

Test meal consumption was calculated by weighing the amount of food consumed and based on the nutritional information provided by the manufacturers. Ad libitum water intake at the test meal was measured by weight (g).

Cumulative energy intake was calculated by adding the caloric content of the fixed breakfast with the calories consumed at the test meal. Caloric compensation, expressed as a percentage, was calculated by subtracting the calories consumed at the test meal following the treatment
from those following the water control divided by the calories in the treatment and multiplied by 100. A caloric compensation of <100% indicates undercompensation meaning that the energy consumed at the test meal plus the energy of the treatment (beverage only) exceeded the energy consumed at the test meal following the control (water), while the opposite is known as overcompensation and is indicated by a score >100%. [94]

5.3.5 Statistical Analyses

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). Two and three-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of treatment, time, sex and their interaction on dependent variables over the study period including changes from baseline for average appetite scores, blood glucose and insulin concentrations and means for FI, cumulative energy intake, water intake and caloric compensation. There were no significant interactions between treatment and sex for any of the variables therefore these results were pooled for both men and women. When there was a significant treatment by time interaction, one-factor ANOVA (PROC MIXED procedure) was followed by Tukey’s post-hoc test to investigate the effect of treatment on changes from baseline for appetite, blood glucose and insulin at each time of measurement and means for FI, cumulative energy intake, water intake and caloric compensation. Post-treatment changes from baseline were calculated from 15-180 min and post-meal changes at 210 min.

Since water was only included as an energy-free control to abide by the guidelines stated in HC’s guidance document for satiety and not PPG, it was only included in the analysis for subjective appetite and FI. Incremental AUCs were calculated for blood glucose and insulin for the cumulative period and separately for each the post-treatment and the post-meal period; post-treatment and post-meal periods were 0-180 min and 180-210 min, respectively, for all variables.
Glucose to insulin ratios were compared among treatments by calculating both the ratios of blood glucose to insulin iAUCs (mmol min L$^{-1}$/µU mL$^{-1}$) post-treatment, post-meal and cumulatively, and the ratios of changes from baseline of blood glucose to insulin at 30 min (mmol min L$^{-1}$/µU mL$^{-1}$). Glucose to insulin ratio measures the efficacy of insulin action [140]. The lower the ratio is, the higher the efficacy of insulin action. Data are presented as mean ± SEM. Statistical significance was set at $p < 0.05$.

5.4 Results

5.4.1 Participant Characteristics

Thirty healthy males and females (sex: 14 males and 16 females; age 64.6±2.4 years; BMI: 25.6±2.5 kg/m$^2$) completed all five sessions. Dropouts include three female participants and one male participant who were excluded due to high fasting blood glucose. Their results were not included in the analysis.

5.4.2 Palatability of treatments

Treatment had an effect on palatability scores ($p < 0.0001$). Water was the least preferred compared to all other treatments which is likely due to it being served alone with no other food. There were no differences between other treatments.

5.4.3 Food and Water Intake

All treatments resulted in similar FI, cumulative energy intake, water intake and caloric compensation and were similarly undercompensated for at the later meal, averaging only 63% (Table 5.2).
5.4.4 Subjective Appetite

Post-treatment (15-180 min) mean appetite changes from baseline were affected by time ($p < 0.0001$), treatment ($p < 0.0001$) and by a time-by-treatment interaction ($p < 0.0001$). There was a decrease in appetite, expressed as changes from baseline, following all treatments compared to water from 0-180 min except at 150 and 180 min where milk was no longer different from water (Fig. 5.1). There were no differences between any other treatments. Post-meal (210 min) mean appetite changes from baseline was affected by treatment ($p < 0.0001$). Water led to lower appetite than all treatments except milk (Fig. 5.1).

Over the entire post-treatment period (15-180 min), yogurt and cheese equally suppressed appetite compared to milk and water but were not different from soy beverage ($p < 0.0001$, Table 5.3). Over the entire post-meal period (210 min), water suppressed appetite compared to all other treatments except milk ($p < 0.0001$, Table 5.3). Cumulative (15-210 min) appetite was the same as the post-treatment period ($p < 0.0001$, Table 5.3).

5.4.5 Blood Glucose Concentrations

Post-treatment (15-180 min) blood glucose mean changes from baseline were affected by time ($p < 0.0001$), treatment ($p < 0.0001$) and by a time-by-treatment interaction ($p = 0.02$). Blood glucose, expressed as changes from baseline, peaked at 45 min and returned to baseline by 180 min for all treatments (Fig. 5.2). Blood glucose was higher following soy beverage compared to yogurt from 30-90 min and higher compared to cheese from 60-120 min. Milk was not different from soy beverage and cheese from 30-60 min and also not different from yogurt from 90-120 min. No differences between treatments were observed at 15, 150 and 180 min. There were no differences between treatments at 210 min in the post-meal period (210 min) (Fig. 5.2).
Over the entire post-treatment period (15-180 min), blood glucose was equally lower for cheese and yogurt compared to soy beverage and milk ($p < 0.0001$; Table 5.3). There were no differences between treatments in the post-meal period (210 min) (Table 5.3). In the cumulative period (15-210 min), soy beverage led to higher blood glucose compared to cheese and yogurt with no differences between milk and cheese ($p < 0.0001$; Table 5.3).

### 5.4.6 Insulin Concentrations

Insulin was measured in only 12 participants (6 males, 6 females). Post-treatment (15-180 min) mean insulin changes from baseline were only affected by time ($p < 0.0001$). Insulin, expressed as changes from baseline, peaked at 30 and 45 min and returned to baseline by 180 min (Fig 5.3). There were no differences between any treatments at any time. There were no differences between treatments in the post-meal period (210 min) (Fig 5.3).

Over the entire post-treatment period (15-180 min), there was a trend showing no differences between yogurt, milk and soy beverage and higher insulin following yogurt compared to cheese in the post-treatment ($p = 0.06$; Table 5.3). There were no differences between treatments in the post-meal (210 min) or cumulative (15-210 min) periods.

### 5.4.7 Blood Glucose to Insulin Ratios

In the post-treatment (15-180 min) period, blood glucose to insulin iAUC was highest for soy beverage compared to yogurt with no differences between any other treatments ($p = 0.01$; Table 5.4). There were no differences between any treatments in the post-meal period (210 min) (Table 5.4). Cumulative (0-210 min) results were the same as the post-treatment. When the ratios for mean changes from baseline at 30 min were considered, all treatments led to similar ratios (Table 5.4).
5.5 Discussion

The results support our hypothesis that when consumed with glycemic carbohydrate, semi-solid and solid dairy products increase satiety and decrease PPG more than liquid forms of dairy products or non-dairy beverages. Yogurt and cheese consumed with glycemic carbohydrate were more effective than milk or soy beverage at reducing appetite and PPG. However, the hypothesis that milk would be more effective than soy beverage was not supported.

This is the first study to report on the effects of dairy products consumed with a source of glycemic carbohydrate by older adults. Since there were no differences in insulin between any of the treatments, differences in blood glucose responses are likely due to insulin-independent mechanisms. Yogurt resulted in a low blood glucose peak and sustained blood glucose levels in the post-treatment period, suggesting delayed gastric emptying mediated by an increase in GI hormones due to protein as described in previous studies [18, 74]. Additionally, Greek yogurt primarily contains casein which is known to clot and form a gel in the stomach [40]. However, the results contrast with a report by El Khoury et al. [62] who found no differences in blood glucose when Greek yogurts containing 23.1 g, 22.2 g and 18.3 g of protein and skim milk containing 16.6 g of protein were compared. In the present study, the yogurt breakfast had almost twice the amount of protein as the milk breakfast suggesting a more potent effect with increased protein.

A trend in the post-treatment period indicated that yogurt resulted in lower blood glucose with the same insulin concentrations as milk and soy beverage (Table 5.3). Additionally, higher efficacy of insulin after the yogurt breakfast compared to the soy beverage breakfast is indicated by the lower blood glucose to insulin iAUC ratio (Table 5.4). Since soy beverage led to the same
ratios as cheese and milk, the higher efficacy of insulin following yogurt is likely due to insulin-independent mechanisms arising from the large amount of protein, as previously described, rather than insulinotropic properties specific to milk proteins.

A second-meal effect on appetite was not detected likely due to the length of time (3 h) between consumption of treatments and the ad libitum lunch which was set to abide by HC’s guidance document for satiety health claims [10]. By 180 min, subjective appetite ratings had already returned to baseline. However, following the ad libitum meal, appetite was lower after water than all treatments except milk. Since there were no differences in FI, this may be due a more heightened sense of satiation after finally eating for the first time that day.

Breakfast meals were isocaloric to remove energy content as a confounder for any differences in satiety. Subjective appetite ratings were affected not only by the protein content of the treatments but as well by food form. Although yogurt contained 10 g more protein than cheese, there were no differences in appetite between them. As previously described, delayed gastric emptying was only observed for yogurt and not cheese therefore a possible explanation in the lack of differences in appetite is the chewing requirements for each food arising from the differences in their food forms. Yogurt, a semi-solid with a gel-like consistency, was eaten with a spoon and required no chewing whereas cheese, a solid, required chewing before swallowing. A recent meta-analysis suggested that increasing chewing time of foods decreases self-reported hunger perhaps due to the stimulation of anorexigenic and the suppression of orexigenic gastrointestinal hormones [154].

The semi-solid yogurt suppressed appetite more than milk but not soy beverage even though the latter were both fluids, suggesting that the composition of soy and not protein content was a
factor. An explanation for the appetite suppression following soy beverage may be due to the carrageenan that is typically added to commercially-available soy beverages as a thickening agent. Carrageenan increases satiety although the amount and type of carrageenan used in this soy beverage was not reported. Furthermore, oro-sensory factors, particularly sweetness increase satiety [147]. Cane sugar (sucrose) is the main sweetener used in this brand of soy beverage. Lactose has a sweetness value of 15 compared with 100 for sucrose, which may explain why milk did not lead to the same appetite suppression as yogurt and cheese despite having more protein than soy beverage.

A limitation of this study was the lack of control treatment for blood glucose as an outcome measure. Water consumed with bread and jam would have provided insightful data about how dairy products affect PPG when consumed with carbohydrate versus consumption of the carbohydrate alone. However, water alone was necessary to serve as the energy-free control outlined in HC’s guidance document for satiety [10]. Studies testing a second-meal effect of glycemia typically use a fixed-amount test meal based on calories per kilogram bodyweight as described in a previous study by Akhavan et al. [18] since ad libitum test meals may cause blunting of treatments effects on insulin. However, since FI was similar for all treatments in the present study, it was able to mimic a fixed-meal design. Therefore, the inability to detect a second-meal effect on insulin and glycemia is likely due to the long delay between the breakfast and the test meal which may have also blunted effects on FI. The period of 180 min was chosen to abide by the HC guidelines for satiety that states that subjective appetite should be measured for at least 3 h for a snack [10]. Finally, since the study was conducted only in healthy individuals, the results cannot be extrapolated to unhealthy populations such as obese, pre-diabetic or diabetic individuals for whom this type of research can ultimately benefit.
5.6 Conclusion

Cheese and yogurt consumed with glycemic carbohydrate at breakfast increase satiety and lower PPG more than milk or a soy beverage and may be effective dietary interventions for appetite and glycemic control in older adults.
Table 5.1
Nutritional composition of treatments and breakfast foods

<table>
<thead>
<tr>
<th></th>
<th>Treatments A</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soy beverage</td>
<td>Cheese</td>
<td>2% Milk</td>
<td>Yogurt</td>
<td>Bread</td>
<td>Jam</td>
</tr>
<tr>
<td>Portion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serving size</td>
<td>250 ml</td>
<td>30 g</td>
<td>250 ml</td>
<td>175 g</td>
<td>75 g</td>
<td>1 g</td>
</tr>
<tr>
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<td>—</td>
<td>220</td>
<td>—</td>
<td>75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Jam B (g)</td>
<td>19.3</td>
<td>27</td>
<td>23.2</td>
<td>23.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nutrients C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
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<td>120</td>
<td>130</td>
<td>130</td>
<td>190</td>
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<td>10</td>
<td>5</td>
<td>3.5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
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<td>6</td>
<td>3</td>
<td>2</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Trans fat (g)</td>
<td>0</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
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<td>25</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>110</td>
<td>240</td>
<td>120</td>
<td>60</td>
<td>300</td>
<td>0.05</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>21</td>
<td>0</td>
<td>12</td>
<td>8</td>
<td>37</td>
<td>0.67</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>4</td>
<td>3</td>
<td>0.57</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>17</td>
<td>6</td>
<td>0.005</td>
</tr>
</tbody>
</table>

A Treatments consumed with 75 g bread and varied amount of jam.
B Amount of jam consumed with each treatment.
C Nutrient content of each product is provided by the manufacturer. Amounts given are per serving size listed of each product.
Table 5.2
Energy intake, cumulative energy intake, water intake and caloric compensation\textsuperscript{A}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Energy intake (kCal)</th>
<th>Water Intake (g)</th>
<th>Caloric Compensation (%)\textsuperscript{D}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test meal\textsuperscript{B}</td>
<td>Cumulative\textsuperscript{C}</td>
<td></td>
</tr>
<tr>
<td>Control\textsuperscript{E}</td>
<td>867.9±57.2</td>
<td>1247.9±57.2</td>
<td>352.3±29.7</td>
</tr>
<tr>
<td>Soy beverage</td>
<td>792.4±53.1</td>
<td>1172.4±53.1</td>
<td>385.1±27.0</td>
</tr>
<tr>
<td>Cheese</td>
<td>760.6±54.4</td>
<td>1140.6±54.4</td>
<td>377.5±27.7</td>
</tr>
<tr>
<td>2% Milk</td>
<td>802.0±49.3</td>
<td>1182.0±49.3</td>
<td>370.0±24.3</td>
</tr>
<tr>
<td>Yogurt</td>
<td>789.3±61.8</td>
<td>1169.3±61.8</td>
<td>395.2±22.9</td>
</tr>
</tbody>
</table>

\textsuperscript{A} All values are means ± SEMs (n = 30). \(P < 0.05\) (treatment effect using proc mixed, Tukey’s post-hoc).

\textsuperscript{B} Energy consumed in an ad libitum meal was measured at 180 min following treatment consumption.

\textsuperscript{C} Energy in breakfast meal (treatment + bread + jam) + energy from meal.

\textsuperscript{D} Caloric compensation = [(kcal consumed at meal after the water control – kcal consumed at the meal after the treatment)/kcal in the treatment] x 100.

\textsuperscript{E} Water (250 mL).
Table 5.3
Effect of treatment on post-treatment, post-meal and cumulative mean changes from baseline for subjective appetite, blood glucose and insulin

<table>
<thead>
<tr>
<th></th>
<th>ControlB</th>
<th>Soy beverage</th>
<th>Cheese</th>
<th>2% Milk</th>
<th>Yogurt</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>3.9±0.8a</td>
<td>-22.2±1.7bc</td>
<td>-23.8±1.8c</td>
<td>-18.1±1.7b</td>
<td>-25.2±1.9c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-61.7±4.7b</td>
<td>-48.1±4.9a</td>
<td>-44.5±5.3a</td>
<td>-52.5±5.0ab</td>
<td>-44.1±4.8a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cumulative</td>
<td>-2.2±1.4a</td>
<td>-25.5±1.7bc</td>
<td>-26.5±1.7c</td>
<td>-22.0±1.8b</td>
<td>-27.9±1.8c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>-</td>
<td>1.6±0.1a</td>
<td>1.2±0.1b</td>
<td>1.4±0.1a</td>
<td>1.1±0.1b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-</td>
<td>1.7±0.2</td>
<td>1.5±0.2</td>
<td>1.5±0.2</td>
<td>1.6±0.2</td>
<td>NSF</td>
</tr>
<tr>
<td>Cumulative</td>
<td>-</td>
<td>1.6±0.1a</td>
<td>1.2±0.1bc</td>
<td>1.4±0.1ab</td>
<td>1.1±0.1c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>-</td>
<td>38.2±4.2</td>
<td>33.2±3.9</td>
<td>40.3±4.5</td>
<td>43.2±4.1</td>
<td>NS (0.06)</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-</td>
<td>47.7±8.9</td>
<td>41.3±7.3</td>
<td>49.2±10.3</td>
<td>36.8±7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative</td>
<td>-</td>
<td>31.2±4.2</td>
<td>27.3±3.9</td>
<td>32.3±4.6</td>
<td>34.8±4.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

A All values are means ± SEMs (n = 30). Means within a row with different superscript letters are significantly different, P < 0.05 (treatment using proc mixed, Tukey’s post hoc).
B Water (250 mL)
C Post-treatment values are mean changes from baseline of all observations before the test meal: 15, 30, 45, 60, 90, 120, 150 and 180 min.
D Post-meal values are mean changes from baseline of all observations after the test meal: 210 min.
E Cumulative values are mean changes from baseline for all observations for the entire session: 15-210 min.
F NS = non significant.
### Table 5.4

Effect of treatment on post-treatment, post-meal and cumulative ratios of blood glucose to insulin iAUC and on ratios of blood glucose to insulin changes from baseline at 30 min

<table>
<thead>
<tr>
<th></th>
<th>Soy beverage</th>
<th>Cheese</th>
<th>2% Milk</th>
<th>Yogurt</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose/insulin</td>
<td>Post-treatment&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>iAUC ratio</td>
<td>Post-meal&lt;sup&gt;D&lt;/sup&gt;</td>
<td>0.08±0.02</td>
<td>0.08±0.02</td>
<td>0.07±0.01</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td></td>
<td>Cumulative&lt;sup&gt;E&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose/insulin</td>
<td>30 min</td>
<td>0.1±0.02</td>
<td>0.2±0.04</td>
<td>0.2±0.03</td>
<td>0.1±0.02</td>
</tr>
<tr>
<td>changes from</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol min L⁻¹/μU min mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>A</sup> All values are means ± SEMs (n = 30). Means within a row with different superscript letters are significantly different, P < 0.05 (treatment using proc mixed, Tukey’s post hoc).

<sup>B</sup> Water (250 mL)

<sup>C</sup> Post-treatment values are mean changes from baseline of all observations before the test meal: 0, 15, 30, 45, 60, 90, 120, 150 and 180 min..

<sup>D</sup> Post-meal values are mean changes from baseline of all observations after the test meal: 210 min.

<sup>E</sup> Cumulative values are mean changes from baseline for all observations for the entire session: 0-210 min.

<sup>F</sup> NS = non significant.
**Figure 5.1**

![Graph showing subjective appetite changes over time](image1)

**Fig. 5.1.** Effect of treatments on average subjective appetite mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 30$).

**Figure 5.2**

![Graph showing blood glucose changes over time](image2)

**Fig. 5.2.** Effect of treatments on blood glucose mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 30$).
Fig. 5.3. Effect of treatments on insulin mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM ($n = 12$).
Chapter 6
General Discussion

These studies are among the first to compare the effects of different dairy products and non-dairy beverages consumed with glycemic carbohydrate on satiety, FI and PPG. The hypothesis that dairy products increase satiety and decrease FI compared to non-dairy beverages was not supported. Also in contrast to the hypothesis, dairy and non-dairy beverages were similar in their attenuation of blood glucose, which was not predicted by the carbohydrate content of the meals. However, semi-solid and solid dairy products decreased PPG compared to dairy and non-dairy beverages through insulin-independent mechanisms supporting the second hypothesis and demonstrating the importance of food form on PPG. Thus, this research shows that the impact of carbohydrates on blood glucose when consumed in a meal cannot be judged by their reported GI alone and provides evidence to support consumption of dairy products within a meal to improve glycemic control.

As previously reported [39], timing of consumption was a main factor in determining FI as evidenced by no effect of breakfast treatments on FI in the second study when the meal was fed at 180 min (Chapter 5) in contrast to when it was fed at 120 min in the first study (Chapter 4). However, in neither study was subjective appetite predictive of later FI which is typical in satiety studies.

Appetite was not determined solely by protein content of the treatments as evidenced by yogurt beverage (6.3 protein) suppressing appetite more than milk (9 g protein) in the first study (Chapter 4) and cheese (7 g protein) suppressing appetite more than milk (9 g protein) in the second study (Chapter 5). Instead, properties of the yogurt beverage such as viscosity and
fermentation, food form and oro-sensory properties affecting appetite (i.e. sweetness) were all factors in determining appetite. Thickening agents added to commercial non-dairy beverages may have also suppressed appetite. Lower post-meal blood glucose following yogurt beverage and milk in the first study (Chapter 4) was correlated with lower FI. Therefore, this is an example of how effective appetite control can have a positive impact on glycemic regulation.

The concept that carbohydrate consumed with a source of protein attenuates PPG compared to carbohydrate alone was clearly demonstrated by this research. As well it provides support for attenuation of blood glucose being mediated by both insulin-independent mechanisms such as delay in gastric emptying as well as insulin-dependent mechanisms such as protein-mediated insulin release. In the first study (Chapter 4), the protein containing dairy and non-dairy beverages consumed with cereal reduced blood glucose compared to water with cereal. The second study (Chapter 5), provided evidence that insulin-independent mechanisms (not measured) were a factor in attenuating blood glucose. Yogurt, with the largest amount of protein (17 g), resulted in a gradual and sustained release of blood glucose and a lower glucose/insulin ratio. When compared to previous studies comparing yogurt and milk [62] and different forms of the same yogurt [79], it is clear that macronutrient content of the treatments rather than food form itself plays a more influential role on PPG.

A secondary purpose of this research was to evaluate the feasibility of HC’s draft guidance documents for satiety and the regulation of PPG [10, 11]. Both experiments were designed to follow the guidelines for both food health claims although there are many contradictions within the guidelines that do not allow for all guidelines to be followed. For example, the satiety document requires the same amount of each treatment to be tested but also states that the test food cannot be higher in energy than the reference food which is often impossible without
modification of the products. Nevertheless, the experiments were designed to follow the guidelines as closely as possible.

First, the participants represented healthy adult populations as required by the documents and a crossover design, which is the recommended method, was used. Secondly, the guidelines state that the amount of treatment tested should be representative of one serving size as stated on the product’s Nutrition Facts Table and consumed at an appropriate time of day. Therefore, one serving of each treatment was tested at breakfast time since that is the meal where milk is most commonly consumed [127]. However, aside from these simple guidelines there were criteria in each guidance document that were conflicting which made it necessary to sacrifice certain aspects for the other. More specifically, the minimum recommended times for measurement of effect following the treatment consumption were conflicting. The satiety guidelines state that appetite should be measured for a minimum of 3 h post-treatment whereas the PPG guidelines recommend that blood glucose be measured for a minimum of 2 h post-treatment. In order for this research to fulfill both, in Experiment 1 (Chapter 4) the post-treatment period was 2 h and in Experiment 2 (Chapter 5) it was extended to 3 h. Two hours is also an optimal post-treatment period if testing for a post-meal effect since it is not too long such that blood glucose and insulin will have returned to baseline prior to the test meal as was the case in Experiment 2. In fact, as indicated by the energy intake results of Experiment 2, it seems 2 h is also more optimal to detect differences in FI. Therefore, although combined studies investigating satiety and PPG are more efficient and cost-effective, this research shows that studies investigating satiety and PPG need to be conducted separately to allow for optimal testing of each outcome and to abide by HC’s guidelines. The criteria in the guidelines which the studies were able and not able to fulfill for satiety and PPG, respectively, are outlined in more detail in Table 6.1a and 6.1b.
Additionally, it is unlikely that dairy products of current composition and use will be able to substantiate comparative health claims with non-dairy beverages with study designs based on HC’s draft guidance documents.

In spite of these limitations their designs, the current research indicates that dairy products consumed in typical serving sizes and as part of a meal can aid in the regulation of appetite and glycemia in both younger and older adults. More importantly, the results demonstrate that dairy products consumed with carbohydrate can attenuate the blood glucose response compared to carbohydrate alone. When compared to non-dairy beverages, soy beverage is the most comparable to dairy beverages because of its protein content. Therefore, soy beverage is a superior choice to almond beverage and perhaps to other non-dairy beverages (e.g. coconut, cashew, rice beverages) for the purposes of glycemic control. Within dairy products, yogurt and cheese are more effective than milk likely due to their high protein and low carbohydrate contents. Therefore, lifelong and frequent consumption of dairy products may be an effective strategy to ensure healthy metabolic outcomes.

6.1 Study Design: Strengths and Limitations

Strengths

A major strength of this research is its relevance and applicability to real life consumption of dairy products and thus, its validity for informing future dietary guidelines and recommendations as well as substantiating food health claims. All dairy products were provided in typical serving sizes and served as part of a breakfast meal which is the most common meal for milk consumption [127]. Furthermore, each study was conducted in two different age populations and in both sexes which indicate that dairy products are effective interventions for younger and older
adults and can be used as a lifelong strategy for glycemic regulation. Finally, all studies were adequately powered.

Limitations

First, although both studies were randomized, any type of blinding was not possible due to the vast differences in taste and appearance between treatments however this is typical of studies of this nature. Second, the ad libitum design of the studies was not the optimal design to test for a second-meal effect on glycemia due to the potential blunting of blood glucose and insulin. A fixed meal design based on kcal per kilogram bodyweight would have been more effective however ad libitum meals are required to measure FI. Third, an inter-study weakness is the inconsistency in study designs. The first study used 120 min as the time between the breakfast meal and ad libitum lunch whereas the second study used 180 min. Additionally, the second study employed a water-only control rather than a water plus carbohydrate breakfast, which would have provided a better comparison for glycemia. However, the second study was modified to better abide by the guidelines outlined in HC’s guidance document for food health claims for satiety. Fourth, these studies were only testing the short-term effect of dairy products on satiety, FI and glycemia and the repeatability of these results over time and long-term effects still remain unclear. Fifth, measurement of other hormones such as gastrointestinal hormones related to appetite and measurement of gastric emptying would have provided a more in-depth understanding of the mechanisms, especially insulin-independent regulations of blood glucose. Finally, although the participants covered two very different age groups, they are only representative of the healthy population and the results of these studies cannot be applied to unhealthy individuals such as those who are obese, pre-diabetic or diabetic.
6.2 Significance and Implications

Dairy products can be used as an effective dietary measure to combat the rising rates of obesity and T2D. Despite the benefits of dairy on metabolic health outcomes, milk consumption has steadily decreased over the past few decades and Canadians are not consuming the daily dairy requirements outlined in Canada’s Food Guide. By providing scientific evidence to support the effectiveness of dairy in the regulation of satiety and PPG, dairy products will receive substantiation for food health claims related to these outcome measures. As a result, their benefits will be communicated through front-of-pack food health claims. Through these health claims, the Canadian public will be informed that dairy products provide benefits beyond essential nutrients and that they can help prevent and manage chronic disease. Additionally, the body of scientific evidence may help inform public health initiatives such as nutritional education and dietary recommendations from government and non-profit regulatory agencies (e.g. Health Canada, Canadian Diabetes Association). Dieticians may also recommend dairy products to at-risk populations such as overweight/obese and diabetic individuals to improve their health. It is hoped that pre-existing negative perceptions surrounding dairy products will be replaced with more accurate information about their healthfulness. As well, through studies comparing dairy and non-dairy alternatives, consumers will realize that most non-dairy alternatives do not offer the same metabolic benefits as dairy. Eventually, milk and dairy consumption in Canada may increase leading to an overall healthier population. This will help reduce healthcare costs related to obesity and T2D. Furthermore, the Canadian dairy industry will see an economic boost from an increased demand for dairy products. Lastly, these short-term dairy studies will form the rationale for conducting longer-term studies with even more ecological validity.
Chapter 7

Conclusion

In conclusion, satiety is higher and blood glucose is lower after high glycemic carbohydrates are consumed at breakfast with dairy products as well as non-dairy beverages, but semi-solid or solid forms may be more efficacious than liquid forms.
Table 6.1a
Compliance to Health Canada’s Draft Guidance Document for Satiety

<table>
<thead>
<tr>
<th>Characterization of Test and Reference Foods</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Include at least 3 experimental preloads: test food, control food or reference food, energy-free control (5.2.2)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Amount of food tested and the reference food should match the serving size as stated in the Nutrition Facts table (5.3.1)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>The test food should be of equal or lower, but never of higher energy content (per serving) than the reference food (5.3.3)</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>The energy-free control, water, should match the test food in organoleptic characteristics only when the latter is in liquid form. For solid foods, the energy-free control preload could be plain water (5.3.4)</td>
<td>✗</td>
<td>✓ ✓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Design and Considerations</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>The study population should be adult individuals who are generally healthy (5.4.1)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>The duration of an effect of foods on subjective satiety measures should be at least 3 hours for a snack, or 4 hours for a meal (5.5.4)</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>It is advisable to avoid buffet style meals (4.1.3)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Study participants should be time-blinded (5.2.9)</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>It is recommended that the effect of the food be tested at least twice, before and at the end of the study period (5.2.1)</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>It is recommended that antecedent levels of energy depletion and physical activity experienced by the study subjects be standardized prior to testing (5.2.10)</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>The basis for sample size calculations should be the ability to detect at least 10% difference in satiety rating, with a statistical significance at ( p \leq 0.05 ) and a power of at least 80% (5.6.1)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Assessment of the satiety response should be done based on the total area under the curve (AUC) (5.6.4)</td>
<td>✗</td>
<td>✗</td>
</tr>
<tr>
<td>It is recommended, whenever possible, to use a mean score comprised of all VAS scales used in a study (5.5.2)</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Table 6.1b  
Compliance to Health Canada’s Draft Guidance Document for the Reduction in Post-Prandial Glycemia

<table>
<thead>
<tr>
<th>Characterization of Test and Reference Foods</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>The test food must be in the same food category or serve a similar dietary role as the reference food with equal or lower amounts of carbohydrate per serving (3.4)</td>
<td>✔️</td>
<td>✔️ ✗</td>
</tr>
<tr>
<td>The amounts of reference and test food given in the study must be consistent with its serving size and intended pattern of consumption (4.1.2)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Design and Considerations</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>The study population should be adult individuals who are generally healthy (4.2.1)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Measurements should be taken for at least 2 hours, with higher frequency (ex: at 15 min intervals) in the first hour, and 30 minutes thereafter (4.3.3)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data on insulin concentrations following the consumption of the test food should be provided (4.3.4)</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>The glycemic and insulminemic responses should be measured as the incremental area under the response curves (iAUC) (4.3.5)</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>
Chapter 8

References

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2. Body mass index, overweight or obese, self-reported, adult, by age group and sex (Percent) [http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health81b-eng.htm]


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Appendices

Appendix 1. Sample Size Calculation

Sample size calculation when testing for the mean of a normal distribution (two-sided alternative), for within subject design, is:

\[ n = [(Z_{1-\alpha/2} + Z_{1-\beta}) \cdot \sigma/\Delta]^2 \]

\( \alpha = 0.05 \), probability of Type I error
\( \beta = 0.20 \), probability of Type II error
\( Z_{0.975} = 1.96 \)
\( Z_{0.80} = 0.84 \)
\( \sigma = 186.2 \text{ kcal} \)
\( \Delta = 157.0 \text{ kcal} \)
\( n = 26 \)

Values were taken from a previous study [139]. \( \sigma \) represents standard deviation, \( \Delta \) represents the minimal difference in food intake between the sugar beverage and water control. \( n \) is the number of subjects required.
Appendix 2. CONSORT Flow Diagram

Experiment 1

Primary Screening (phone/email) (n= 111)

Excluded (n= 51)
- BMI = 22
- Trying to lose/gain weight = 8
- Protein powder use = 6
- No longer interested = 4
- Age = 3
- Elite/training athlete = 2
- Irregular menstrual cycles = 2
- Excludes dairy = 1
- Doesn’t want to give blood = 1
- Smokes = 1
- Recreational drug use = 1

Secondary Screening (in-person) (n= 60)

Excluded (n= 31)
- Eating Habits Score = 15
- Scheduling issues = 7
- BMI = 4
- No longer interested = 3
- Smokes = 1
- Trying to gain weight = 1

Randomized (n= 29)

Excluded (n= 3)
- Participation in another study = 2
- Scheduling = 1

Analysed (n= 26)
Experiment 2

Enrollment

Primary Screening (phone/email) (n= 119)

Excluded (n= 63)
- BMI = 19
- Scheduling issues = 10
- No longer interested =9
- Vegetarian = 6
- Dislike of study foods = 4
- Lactose intolerant = 3
- Recent surgery/illness = 3
- Age = 3
- Diabetic/pre-diabetic = 2
- Doesn’t want to give blood = 2
- Unstable appetite = 1
- Trying to lose weight = 1

Secondary Screening (in-person) (n= 56)

Excluded (n= 22)
- Eating Habits Score = 15
- BMI = 4
- Diet-related issues = 2
- Doesn’t want to give blood = 1

Allocation

Randomized (n= 30)

Excluded for high fasting blood glucose (n= 4)

Analysis

Analysed (n= 26)
Appendix 3. Experimental Protocols

Experiment 1

Experiment 2

Blood Glucose
Visual Analogue Scales
Insulin
## Appendix 4. Beverage Ingredients

<table>
<thead>
<tr>
<th>Beverages</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt Beverage (Experiment 1)</td>
<td>Skim milk, water, sugar, cream, milk protein concentrate, bacterial cultures, natural flavour, vitamin D3</td>
</tr>
<tr>
<td>Yop, Vanilla-flavoured</td>
<td></td>
</tr>
<tr>
<td>Soy Beverage (Experiment 1)</td>
<td>Filtered water, whole organic soybeans, organic cane sugar, sea salt, carrageenan, natural flavours, acidity regulator (sodium bicarbonate), anti-foaming agent (organic soybean oil, silicon dioxide)</td>
</tr>
<tr>
<td>Silk, Original Organic</td>
<td></td>
</tr>
<tr>
<td>Almond Beverage (Experiment 1)</td>
<td>Almond base (filtered water, almonds), cane sugar, sea salt, locust bean gum, sunflower lecithin, gellan gum</td>
</tr>
<tr>
<td>Silk, Original</td>
<td></td>
</tr>
<tr>
<td>Soy Beverage (Experiment 2)</td>
<td>Filtered water, soy protein (isolate, whole soybeans), cane sugar, corn syrup solids, fructose, canola oil, tricalcium phosphate, potassium citrate, natural vanilla flavour, brown rice syrup, dipotassium phosphate, magnesium phosphate, salt, carrageenan</td>
</tr>
<tr>
<td>So Good, Vanilla</td>
<td></td>
</tr>
</tbody>
</table>


Appendix 5. Nutritional Composition of Test Meals

Experiment 1

Pizza* composition data from manufacturer

<table>
<thead>
<tr>
<th>Nutritional Information per pizza</th>
<th>Three Cheese</th>
<th>Pepperoni</th>
<th>Deluxe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>81</td>
<td>87</td>
<td>92</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>180</td>
<td>180</td>
<td>170</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>23</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*McCain Foods: Deep and Delicious, 5” Pizza

Experiment 2

Rice*, meatball† and tomato sauce§ composition data from manufacturers

<table>
<thead>
<tr>
<th>Nutritional Information per amount included in one bowl</th>
<th>Rice</th>
<th>Meatballs</th>
<th>Tomato Sauce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>250</td>
<td>108</td>
<td>121.5</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>444</td>
<td>303.2</td>
<td>80</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>5.6</td>
<td>22.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>344.4</td>
<td>5.7</td>
<td>13</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>11.1</td>
<td>17.1</td>
<td>2</td>
</tr>
</tbody>
</table>

*Uncle Ben’s Bistro Express Basmati Rice
†President’s Choice Italian Beef Meatballs
§Ragu Original Tomato Sauce
Appendix 6. Recruitment Materials

Experiment 1

Poster

![Poster](image-url)

**Male and Female Participants Needed for Dairy and Cereal Study!**

You may be eligible if you are...
- 20-30 years old
- non-smoking
- healthy

Study involves:
- 1 in-person screening
- 5 study sessions

**FINANCIAL COMPENSATION & FOOD PROVIDED**

If interested, contact 416 978 3700 or dairyandcerealstudy@gmail.com
Experiment 2

Poster

OLDER PARTICIPANTS NEEDED FOR DAIRY NUTRITION STUDY!

You may be eligible if you are…
- 60-70 years old
- non-smoking
- non-diabetic

**FINANCIAL COMPENSATION PROVIDED**

If interested, call 416 399 2047 or email dairybreakfaststudy@gmail.com

Approved by the University of Toronto Research Ethics Board (Protocol #11509)

Toronto Star Newspaper Posting

<table>
<thead>
<tr>
<th>OLDER PARTICIPANTS NEEDED FOR DAIRY NUTRITION STUDY!</th>
</tr>
</thead>
<tbody>
<tr>
<td>You may be eligible if: 60-70 years old, non-smoking, non-diabetic</td>
</tr>
<tr>
<td>Financial compensation provided</td>
</tr>
<tr>
<td>Call: 416 399 2047 or Email: <a href="mailto:dairybreakfaststudy@gmail.com">dairybreakfaststudy@gmail.com</a></td>
</tr>
</tbody>
</table>
Appendix 7. Information and Consent Forms

Experiment 1

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The effect of commercially-available dairy and non-dairy alternatives when consumed with a high glycemic cereal on subjective appetite ratings and post-prandial glycemia in healthy young adults

Information Sheet and Consent Form

Investigators: Dr. G. Harvey Anderson, Professor
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-1832
Email: harvey.anderson@utoronto.ca

Marron Law, MSc. Student
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Pedro Huot, PhD. Candidate
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-6894
Email: pedro.huot@mail.utoronto.ca

Khulood Al-Dabous, Research Assistant
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-6894
Email: k.aldabous@gmail.com
Funding Source:

Funding for this project is provided by Dairy Farmers of Canada.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood glucose. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Dairy products can possibly be used to increase satiety, decrease food intake, and control glycemia and other metabolic factors. However, non-dairy alternatives such as soy and almond milks are rising in popularity. Therefore, it is important to investigate if these products lead to similar effects on satiety and glycemia as dairy products. The information obtained from this study will potentially be used as evidence to promote the importance of incorporating dairy products into the daily diet as a means to achieve healthy body weight and metabolic profile.

The purpose of this research project is to compare the effects of consuming commercially-available dairy products and non-dairy alternatives on satiety and glycemia.

This study will have 30 participants, both male and female.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be required to come in for 5 study sessions over a minimum period of 5 weeks. At each session, you will consume 1 of 5 treatments. The treatments are as follows: 1) 1% milk (Neilson, St. Laurent, Quebec), 2) soy milk (Silk Original, Broomfield, Colorado), 3) almond milk (Silk Original, Broomfield, Colorado), 4) yogurt beverage (Yop – General Mills, Mississauga, Ontario), 5) water. All treatments are to be consumed with Cheerios cereal (General Mills, Mississauga, Ontario). At each study session, you will also be required to consume a pizza lunch (McCain Foods, Toronto, Ontario).

Eligibility:

To participate in this study, you must have a body mass index between 20.0 and 24.9 kg/m² and be between the ages of 20 and 30 years old. You must also be a non-smoker and you cannot be taking any medications. You are required to inform study researchers if there are any changes to these criteria during the study. The study will take place in the Department of Nutritional Sciences, Rooms 334, 331 and 331A, FitzGerald Building, located at 150 College Street, Toronto, ON M5S 3E2.

Procedure:

To determine your eligibility for the study, you will be asked to fill out questionnaires, which will ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your
eating habits, and your ability and willingness to consume all foods provided in the study (i.e. likeability, allergies, etc.). Your height and weight will be measured.

If you are eligible, you will be scheduled for 5 study sessions, each lasting approximately 3 hours. You will be scheduled to come in for study sessions at a chosen time between 8:30 and 10 am on the same day each week.

The day before each study session, you will be asked to refrain from alcohol, heavy exercise, and to keep a 24-hour food record. You will be expected to eat similarly the evening before each study session. For each study session, you will be asked not to eat for 12 hours before arriving at the Department of Nutritional Sciences at the University of Toronto. However, you can drink water up to 1 hour before meeting with us.

At each session, you will be asked to consume the assigned treatment (400 mL) along with Cheerios cereal (~70 g) and a glass of water (125 mL). Ten times during each session, for a total of 50 times over the whole study, you will be asked to provide a small drop of blood by fingerprick. Blood will be sampled before eating the treatment (0 minutes) and at 15, 30, 45, 60, 75, 90, 120, 140, and 170 minutes. You will be asked to fill out visual analogue scales (VAS) measuring your appetite and physical comfort as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. You will be served an all-you-can-eat pizza lunch 120 minutes after you eat the treatment. Each session will last up to 3 hours.

**Time and Activity Schedule for Study Session (example)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>Arrive at the Department of Nutritional Sciences</td>
</tr>
<tr>
<td>8:35</td>
<td>Fill in Sleep, Stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>8:45 – 8:55</td>
<td>Eat the treatment</td>
</tr>
<tr>
<td>8:55-10:55</td>
<td>Blood sampling and VAS questionnaires at 15, 30, 45, 60, 75, 90 and 120 minutes</td>
</tr>
<tr>
<td>10:55-11:15</td>
<td>All-you-can-eat pizza meal</td>
</tr>
<tr>
<td>11:15-11:45</td>
<td>Blood sampling and VAS questionnaires at 140 and 170 minutes</td>
</tr>
</tbody>
</table>

**VAS:** Visual analogue scale

**Voluntary Participation and Early Withdrawal:**

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at any time without any consequences and you will be paid for sessions completed.

**Early Termination:**
Risks:

All of the foods and beverages that you will be asked to consume are commercially-available from the grocery store, prepared hygienically in the kitchen and present minimal risk.

After the overnight fast you may feel faint or dizzy, however the risk of this is minimal.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your fingerprick blood samples. The investigator will help you. To make sure that you are not exposed to another person’s study belongings, we will ask you to sit away from other study participants. We will be collecting your fingerprick blood samples by using disposable lancets. We will swab your finger with alcohol before and after each fingerprick and will use a new sterile lancet each time.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. You will be provided with your own finger prick gun for the entire study.

A total of 10 finger pricks will be conducted per session and may result in some discomfort.

There is very little risk of infection. Before the finger is pricked the area is cleaned with an alcohol swab. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood glucose is measured.

There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. All treatments as well as pizza are freshly prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer’s instructions immediately before you are served.

Benefits:

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and if they are not normal you will be told and advised to talk to your doctor. The foods and drinks will be provided for free and you will be financially compensated.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator’s office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.
Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product. You will not share in any way from the possible gains or money made by commercial application of findings.

Alternative Treatment/Therapy:

N/A

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $40 per session. You will also be given $6 per session for travel (bus, subway). If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

Rights of Subjects:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information, please do not hesitate to ask the investigators for further clarification.

Dissemination of findings:

A summary of results will be made available to you to pick up after the study is completed.
Copy of informed consent for participant:

You are given a copy of this informed consent to keep for your own records.

Consent:

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

___________________                      ______________                    _____________
Participant Name                                 Signature                                           Date

___________________                      ___________________                    _____________
Witness Name                                     Signature                                           Date

___________________                      ___________________                    _____________
Investigator Name                               Signature                                           Date
The effect of dairy and non-dairy breakfasts on subjective appetite ratings and post-meal blood glucose in older adults

Information Sheet and Consent Form

Investigators: Dr. G. Harvey Anderson, Professor
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-1832
Email: harvey.anderson@utoronto.ca

Marron Law, Msc. Student
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-6894
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Dr. Muhammad Umair Arshad, Post-Doctoral Fellow
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-6894
Email: umair.arshad@utoronto.ca
Funding Source:

Funding for this project is provided by Dairy Farmers of Canada.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood glucose. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Dairy products can possibly be used to increase satiety, decrease food intake, and control blood glucose and positively affect other metabolic factors. Therefore, it is important to investigate the effect of dairy products on these health outcomes when consumed in a manner reflective of real life (such as part of a breakfast meal). The information obtained from this study will potentially be used as evidence to promote the importance of incorporating dairy products into the daily diet as a means to achieve a healthy body weight and metabolic profile.

The purpose of this research project is to compare the effects of consuming dairy and non-dairy breakfasts on satiety and glycemia.

This study will have 30 participants, both male and female.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be required to come in for 5 study sessions over a minimum period of 5 weeks. At each session, you will consume 1 of 5 treatments. The treatments are as follows: 1) 2% milk (Neilson, St. Laurent, Quebec), 2) 2% Greek yogurt (Danone, Boucherville, Quebec), 3) cheddar cheese (Armstrong, St. Laurent, Quebec), 4) soy beverage (So Good, Vancouver, BC, Canada), and 5) water. All treatments are to be consumed as part of a breakfast meal with white toast (Wonder Bread Canada, Toronto, Ontario) and strawberry jam (Smucker’s, Orrville, Ohio), with the exception of the water treatment which will be consumed alone.

Eligibility:

To participate in this study, you must have a body mass index between 18.5 and 29.0 kg/m² and be between the ages of 60 and 70 years old. You must also be a non-smoker and you cannot be taking any medications that can affect appetite and metabolism. You are required to inform study researchers if there are any changes to these criteria during the study. The study will take place in the Department of Nutritional Sciences, Rooms 331 and 342 in the FitzGerald Building, located at 150 College Street, Toronto, ON M5S 3E2.

Procedure:

To determine your eligibility for the study, you will be asked to fill out questionnaires, which will ask questions about your age, if you smoke, exercise, your health, if you are on any medications, your eating
habits, and your ability and willingness to consume all foods provided in the study (i.e. likeability, allergies, etc.). Your height and weight will be measured.

If you are eligible, you will be scheduled for 5 study sessions, each lasting approximately 3.5 hours. You will be scheduled to come in for study sessions at a chosen time between 8 am and 10 am on the same day each week.

The day before each study session, you will be asked to refrain from alcohol and heavy exercise. You will be expected to eat similarly the evening before each study session. For each study session, you will be asked not to eat for 12 hours before arriving at the Department of Nutritional Sciences at the University of Toronto. However, you can drink water up to 1 hour before meeting with us.

At each session, you will be asked to consume the assigned treatment along with white toast, strawberry jam, and water. Ten times during each session, for a total of 50 times over the whole study, you will be asked to provide a small drop of blood by fingerprick. Blood will be sampled before eating the treatment (0 minutes) and at 15, 30, 45, 60, 90, 120, 150, 180 and 210 minutes. You will be asked to fill out visual analogue scales (VAS) measuring your appetite and physical comfort as well as the palatability (pleasantness) of the foods throughout the study sessions. You will be served an all-you-can-eat lunch consisting of a rice dish (white rice, meatballs, and tomato sauce) 180 minutes after you eat the treatment. Each session will last up to 3.5 hours.

**Time and Activity Schedule for Study Session (example)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>Arrive at the Department of Nutritional Sciences</td>
</tr>
<tr>
<td>8:35</td>
<td>Fill in Sleep, Stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>8:40 – 8:50</td>
<td>Eat the treatment</td>
</tr>
<tr>
<td>8:55-11:40</td>
<td>Blood sampling and VAS questionnaires at 15, 30, 45, 60, 90, 120, 150 and 180 minutes</td>
</tr>
<tr>
<td>11:40-12:00</td>
<td>All-you-can-eat meal</td>
</tr>
<tr>
<td>12:10</td>
<td>Blood sampling and VAS questionnaires at 200 minutes</td>
</tr>
</tbody>
</table>

**VAS:** Visual analogue scale

**Voluntary Participation and Early Withdrawal:**

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at any time without any consequences and you will be paid for sessions completed.
**Early Termination:**

Not applicable.

**Risks:**

All of the foods and beverages that you will be asked to consume are commercially-available from the grocery store, prepared hygienically in the kitchen and present minimal risk.

After the overnight fast you may feel faint or dizzy, however the risk of this is minimal.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your fingerprick blood samples. The investigator will perform the fingerpricks for you. However, you may request to perform your own fingerpricks. To make sure that you are not exposed to another person’s study belongings, we will ask you to sit away from other study participants. We will be collecting your fingerprick blood samples by using single-use, disposable lancets. We will swab your finger with alcohol before and after each fingerprick and will use a new sterile lancet each time.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. You will be provided with your own finger prick gun for the entire study.

A total of 10 finger pricks will be conducted per session and may result in some discomfort.

There is very little risk of infection. Before the finger is pricked the area is cleaned with an alcohol swab. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood glucose is measured.

There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. All treatments as well as the rice dish are freshly prepared at the time of your session. The components of the rice dish are cooked accordingly to the manufacturer’s instructions immediately before you are served.

**Benefits:**

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and if they are not normal you will be told and advised to talk to your doctor. The foods and drinks will be provided for free and you will be financially compensated.

**Confidentiality and Privacy:**

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator’s office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer
records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

**Publication of Results:**

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

**Possible Commercialization of Findings:**

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product. You will not share in any way from the possible gains or money made by commercial application of findings.

**Alternative Treatment/Therapy:**

N/A

**New Findings:**

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

**Compensation:**

You will be paid $56 per session which includes $6 for travel (bus, subway). If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

**Injury Statement:**

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

**Rights of Subjects:**

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information, please do not hesitate to ask the investigators for further clarification.
**Dissemination of findings:**

A summary of results will be made available to you to pick up after the study is completed.

**Copy of informed consent for participant:**

You are given a copy of this informed consent to keep for your own records.

**Consent:**

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the in-person screening and the study, and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

___________________                      ___________________                    _____________
Participant Name                      Signature                      Date

___________________                      ___________________                    _____________
Witness Name                          Signature                      Date

___________________                      ___________________                    _____________
Investigator Name                     Signature                      Date
Appendix 8. Screening Questionnaires

Primary (Phone/Email) Screening Questionnaire
Secondary (In-Person) Screening Questionnaire
Sleep Habits Questionnaire
Eating Habits Questionnaire
Food Acceptability and Frequency Questionnaire
Participant Eligibility Summary
8.1 Primary (Phone/Email) Screening Questionnaire

Experiment 1

The Dairy and Cereal Study:

PRIMARY SCREENING QUESTIONNAIRE

Study Coordinator:

Date: ___________________________ Time: ___________________________

How did you learn about the study? Poster □ Friend □

I am now going to provide you with a little more detail about the study to ensure your interest before proceeding to a preliminary screening questionnaire to determine your eligibility. This will take approximately 10 minutes. Feel free to stop me at any time if you have any questions.

The purpose of the Dairy and Cereal Study is to test the effects of commercially-available dairy products and dairy alternatives on satiety, which is the feeling of fullness, appetite, and glycemia. A total of 30 participants, both male and female, will be invited to participate. Participants will be required to come into the Department of Nutritional Sciences, located in the Fitzgerald Building at 150 College St., on 6 separate occasions; once for an in-person screening lasting approximately 30 minutes, and 5 times for study visits lasting approximately 4 hours. During these study visits, participants will consume a provided breakfast and lunch. Participants will periodically rate their feelings and perceptions, and provide blood samples through finger-prick for the analysis of blood glucose and insulin.

Are you comfortable with providing blood samples through fingerprick? Yes □ No □

Participant Name: ___________________________ Screening ID: ___________________________

Phone #: ___________________________ Email: ___________________________

Date of Birth (dd/mm/yyyy): ___________________________ Age: ___________________________

Preferred method of contact: ___________________________
1. Are you male or female?      MALE □      FEMALE □

2. What is your age?            ______________

3. What is your height?         ______________

4. What is your weight?         ______________

Calculated BMI:                ______________

5. Do you have any medical conditions (e.g. diabetes, lactose intolerance
Celiac’s disease, Crohn’s, cardiovascular disease)   YES □     NO □

    If YES, please specify:___________________________________________________

6. Do you use any prescription or over-the-counter medications? YES □     NO □

    If YES, please specify:__________________________________________________

7. Do you use any protein powders/supplements? YES □     NO □

    If YES, please specify:__________________________________________________

8. Do you smoke?                YES □     NO □

9. Do you use recreational drugs? YES □     NO □

10. Do you have any anaphylactic or life-threatening allergies? YES □     NO □

    If YES, please specify:__________________________________________________

***FOR FEMALES ONLY***

11. Are you currently, recently, or planning on becoming pregnant? YES □     NO □

12. Are you currently breastfeeding? YES □     NO □

13. Do you have regular monthly menstrual cycles? YES □     NO □

************
14. Are you currently trying to lose or gain weight?  

YES □  NO □

*Elite athletes are defined as anyone currently competing as a varsity player (individual or team), a professional player or a national or international level player. Training athletes are participating in intense practice and exercise for individual or team events.*

15. Based on this definition, do you consider yourself an elite or training athlete?  

YES □  NO □

16. Do you follow any special diet? (i.e. vegetarian, vegan, etc.)  

YES □  NO □  

If YES, please specify:  
_______________________________________________

17. Do you exclude dairy products from your diet?  

YES □  NO □

18. In one week, how many days do you eat breakfast?  

_______________

*This study will require participants to consume milk, soy milk, almond milk, and yogurt along with Cheerios cereal.*

19. Are you comfortable with consuming all these foods?  

YES □  NO □  

If NO, please specify:  
__________________________________________________

*This study will also require participants to consume pizza.*

20. Do you like pizza?  

YES □  NO □

21. Would you be willing to come in to the Department of Nutritional Sciences for an in-person screening visit?  

YES □  NO □

22. If eligible for the study, you will be asked to come in to the Department of Nutritional Sciences for 5 separate study visits. Study visits will start at a chosen time between 8-10 am and last approximately 5 hours. There is a minimum one-week period between each visit so the study will take a minimum of 5 weeks to complete. Would your schedule be able to accommodate this?  

YES □  NO □
The purpose of the Dairy Breakfast Study is to test the effects of dairy and non-dairy breakfasts on satiety (the feeling of fullness after eating) and blood glucose. A total of 30 participants, both male and female, will be invited to participate. Participants will be required to come into the Department of Nutritional Sciences, located in the Fitzgerald Building at 150 College St., on 6 separate occasions; once for an in-person screening lasting approximately 30 minutes, and 5 times for study sessions lasting approximately 3.5 hours. During these study sessions, participants will consume a provided breakfast and lunch. Participants will periodically rate their feelings and perceptions, and provide 10 blood samples through finger-prick for the analysis of blood glucose and insulin.

Are you comfortable with providing blood samples through finger-prick?

Yes □ No □
Please complete the questionnaire below.
You do not have to answer questions you are uncomfortable with.

1. Are you male or female?  
   MALE □  FEMALE □

2. What is your age?  
   ___________________

3. What is your height?  
   ___________________

4. What is your weight?  
   ___________________
   Calculated BMI: _______________

MEDICAL AND HEALTH ASSESSMENT

5. Do you have any of the following:
   a) diabetes?  
      YES □  NO □
   b) any medical conditions or diseases? (e.g. cardiovascular disease, fatty liver disease, etc.)  
      YES □  NO □
      If YES, please specify:______________________________________________________________
   c) any gastrointestinal diseases? (e.g. Celiac’s disease, Crohn’s, etc)  
      YES □  NO □
      If YES, please specify:______________________________________________________________
   d) any medical disorders? (e.g. high blood pressure, high fasting blood sugar, high cholesterol, high triglycerides)  
      YES □  NO □
      If YES, please specify:______________________________________________________________

6. Have you had any of the following:
   a) any major health event in the past? (e.g. heart attack, stroke, cancer, etc.)  
      YES □  NO □
      If YES, please specify:______________________________________________________________
   b) hospitalization within the past year (12 months)?  
      YES □  NO □
      If YES, please explain:______________________________________________________________
   c) surgeries within the past year (12 months)?  
      YES □  NO □
      If YES, please specify:______________________________________________________________
7. Do you use any prescription or over-the-counter medications? YES □ NO □
If YES, please specify: ______________________________________________________________

8. Do you have any food allergies? YES □ NO □
If YES, please specify: ______________________________________________________________

9. Do you have any anaphylactic or life-threatening allergies? YES □ NO □
If YES, please specify: ______________________________________________________________

***FOR WOMEN ONLY***

10. Have you undergone menopause? YES □ NO □
If YES, has it been for at least 1 year? (i.e. no periods within the past 12 months) YES □ NO □

11. Are you taking any hormonal treatments? YES □ NO □
If YES, has it been for at least 1 year? YES □ NO □

**************

12. Do you smoke? YES □ NO □

13. Are you currently trying to lose or gain weight? YES □ NO □

14. Do you follow any special diet? (i.e. vegetarian, vegan, etc.) YES □ NO □
If YES, please specify: ______________________________________________________________

15. Are you lactose intolerant? YES □ NO □

16. Do you exclude dairy products from your diet? YES □ NO □

17. Do you suffer any gastrointestinal discomfort after consuming dairy products? YES □ NO □

18. In one week, how many days do you eat breakfast? ____________

This study will require participants to consume milk, plain Greek yogurt, cheddar cheese, soy milk, toast, strawberry jam.

19. Are you comfortable with consuming these foods? YES □ NO □
If NO, please specify:___________________________________________________________________

This study will also require participants to consume rice with meatballs and tomato sauce.

20. Are you comfortable with consuming these foods? YES □ NO □

If NO, please specify:___________________________________________________________________

21. Would you be willing to come in to the Department of Nutritional Sciences for an in-person screening visit? YES □ NO □

22. If eligible for the study, you will be asked to come in to the Department of Nutritional Sciences for 5 separate study sessions. Study sessions will start at a chosen time between 8-10 am and last approximately 3.5 hours. There is a minimum one-week period between each visit so the study will take a minimum of 5 weeks to complete. Would your schedule be able to accommodate this? YES □ NO □
8.2 Secondary (In-Person) Screening Questionnaire

Experiment 1

The Dairy and Cereal Study:
IN-PERSON SCREENING QUESTIONNAIRE

Participant Initials: _________________________________

Date: ____________________      Time: __________

Thank you very much again for your interest in the study. The purpose of this questionnaire is to further determine your eligibility and to ensure your safety as a participant in the study.

Please note: All information provided in this questionnaire will be kept strictly confidential.
Please complete the questionnaire below.
You do not have to answer questions you are uncomfortable with.

1. How would you describe your general health?
   POOR □    GOOD □    VERY GOOD □    EXCELLENT □

2. Do you have any of the following:
   a) Diabetes?  YES □    NO □
   b) Lactose intolerance?  YES □    NO □
   c) Celiac’s disease?  YES □    NO □
   d) Any other medical or gastrointestinal conditions?  YES □    NO □

   If YES, please specify:____________________________________________________

3. Do you smoke?  YES □    NO □

4. Do you use recreational drugs?  YES □    NO □

5. Do you use any protein powders/supplements?  YES □    NO □

6. Do you use any prescription or over-the-counter medications?  YES □    NO □

   If YES, please complete the table below:

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Reason for Use</th>
<th>Frequency</th>
<th>Length of Use</th>
</tr>
</thead>
</table>

7. Do you use any dietary supplements or natural health products (e.g. multivitamins, omega-3, fibre, probiotics)?

   If YES, please complete the table below:  YES □    NO □

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Reason for Use</th>
<th>Active Ingredient</th>
<th>Frequency</th>
<th>Length of Use</th>
</tr>
</thead>
</table>

121
8. This study requires that participants maintain their usual medication and dietary supplement routine for the duration of the study. Would you be comfortable with this?  
YES □  NO □

***FOR FEMALES ONLY***

9. Are you currently, recently, or planning on becoming pregnant?  
YES □  NO □

10. Are you currently on any hormonal contraceptives? (e.g. pill, ring, IUD, patch)  
YES □  NO □

11. This study requires participants maintain their hormonal contraceptive routine for the duration of the study. Would you be comfortable with this?  
YES □  NO □

12. Impaired insulin sensitivity has been observed in the luteal phase of the menstrual cycle. Therefore, this study requires participants track and discuss their menstrual cycle with study coordinators. Would you be comfortable with this?  
YES □  NO □

13. Do you have regular monthly menstrual cycles?  
YES □  NO □

14. Please circle the FIRST day of your last menstrual cycle AND the FIRST day of your next expected menstrual cycle.

<table>
<thead>
<tr>
<th>January</th>
<th>February</th>
</tr>
</thead>
<tbody>
<tr>
<td>S M T W T F S</td>
<td>S M T W T F S</td>
</tr>
<tr>
<td>1 2 3 4 5 6 7</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>8 9 10 11 12 13 14</td>
<td>8 9 10 11 12 13 14</td>
</tr>
<tr>
<td>15 16 17 18 19 20 21</td>
<td>15 16 17 18 19 20 21</td>
</tr>
<tr>
<td>22 23 24 25 26 27 28</td>
<td>22 23 24 25 26 27 28</td>
</tr>
<tr>
<td>29 30 31</td>
<td>29 30 31</td>
</tr>
</tbody>
</table>

****************
15. Please complete the following table about your participation in athletics, physical activity and exercise.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (Days/Week)</th>
<th>Duration (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

16. This study requires participants refrain from vigorous exercise 24 hours before each study visit (a total of 5 occasions). Would you be comfortable with this?  

YES □  NO □

17. Are you currently on a diet or exercise regime for weight loss or weight gain?  

YES □  NO □

18. This study requires participants to maintain their body weight for the duration of the study. Would you be comfortable with this?  

YES □  NO □

19. How many alcoholic drinks do you consume per week?  
(1 drink = 12 oz beer, 5 oz wine, 1.5 oz hard liquor)  

_____________

20. This study requires participants refrain from alcohol consumption 24 hours before each study visit (a total of 5 occasions). Would you be comfortable with this?  

YES □  NO □

21. In one week, how many days do you consume caffeinated beverages?  
(e.g. coffee, tea, pop, energy drinks)  

_____________

22. How many caffeinated beverages do you consume per day?  

_____________

23. Do you follow any special diet? (i.e. vegetarian, vegan, etc.)  

YES □  NO □

If YES, please specify: __________________________________________________________________________

24. Do you exclude dairy, soy, or almond products from your diet?  

YES □  NO □

25. This study requires participants to come in for 5 separate study visits, each lasting approximately 3 hours. Study visits will start between 8:30-10 am. There is a minimum one-week period
between each visit so the study will take a minimum of 5 weeks to complete. Can your schedule accommodate this?   YES □   NO □

26. What time in the morning (between 8:30-10 am) would you prefer to start?   ______________

27. Please indicate which day(s) of the week you would be able to attend a study visit.

MON □    TUES □    WED □    THURS □    FRI □    SAT □    SUN □

28. Are you currently participating in any other research study?

   If YES, please specify: ______________________________________________________

   Thank you!
Experiment 2

The Dairy Breakfast Study:
IN-PERSON SCREENING QUESTIONNAIRE

Participant Initials: _________________________________
Date: ____________________      Time: ________

Thank you very much again for your interest in the study. The purpose of this questionnaire is to further determine your eligibility and to ensure your safety as a participant in the study.

Please note: All information provided in this questionnaire will be kept strictly confidential.
Please complete the questionnaire below.
You do not have to answer questions you are uncomfortable with.

1. How would you describe your general health?

   POOR □ GOOD □ VERY GOOD □ EXCELLENT □

2. Do you have any of the following:

   a) Diabetes? YES □ NO □
   b) Lactose intolerance? YES □ NO □
   c) Celiac’s disease? YES □ NO □
   d) Any other medical or gastrointestinal conditions?
      YES □ NO □
      If YES, please specify:____________________________________________________

3. Do you smoke? YES □ NO □

4. Do you use any prescription or over-the-counter medications?
   YES □ NO □
   If YES, please complete the table below:

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Reason for Use</th>
<th>Frequency</th>
<th>Length of Use</th>
</tr>
</thead>
</table>

5. Do you use any dietary supplements or natural health products (e.g. multivitamins, omega-3,
fibre, probiotics)?

   YES □ NO □
   If YES, please complete the table below:

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Reason for Use</th>
<th>Active Ingredient</th>
<th>Frequency</th>
<th>Length of Use</th>
</tr>
</thead>
</table>
6. This study requires that participants maintain their usual medication and dietary supplement routine for the duration of the study. Would you be comfortable with this? YES □ NO □

7. Please complete the following table about your participation in athletics, physical activity and exercise.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Frequency (Days/Week)</th>
<th>Duration (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

8. This study requires participants refrain from vigorous exercise 24 hours before each study visit (a total of 5 occasions). Would you be comfortable with this? YES □ NO □

9. Are you currently on a diet or exercise regime for weight loss or weight gain? YES □ NO □

10. This study requires participants to maintain their body weight for the duration of the study. Would you be comfortable with this? YES □ NO □

11. How many alcoholic drinks do you consume per week? (1 drink = 12 oz beer, 5 oz wine, 1.5 oz hard liquor) ______________

12. This study requires participants refrain from alcohol consumption 24 hours before each study visit (a total of 5 occasions). Would you be comfortable with this? YES □ NO □

13. In one week, how many days do you consume caffeinated beverages? (e.g. coffee, tea, pop, energy drinks) ______________

14. How many caffeinated beverages do you consume per day? ______________

15. Do you follow any special diet? (i.e. vegetarian, vegan, etc.) YES □ NO □

   If YES, please specify: __________________________________________________________________________

16. This study requires participants to come in for 5 separate study visits, each lasting approximately 3 hours. Study visits will start between 8:30-10 am. There is a minimum one-week period
between each visit so the study will take a minimum of 5 weeks to complete. Can your schedule accommodate this?  YES □  NO □

17. What time in the morning (between 8:30-10 am) would you prefer to start?  ________________

18. Please indicate which day(s) of the week you would be able to attend a study visit.

MON □  TUES □  WED □  THURS □  FRI □  SAT □  SUN □

19. Are you currently participating in any other research study?

If YES, please specify: ________________________________________________________________

Thank you!
8.3 Sleep and Breakfast Habits Questionnaire

1. At what time do you normally go to sleep?
   
   During the week: ___________________  Weekends/days off: _________________

2. At what time do you normally wake up?
   
   During the week: ___________________  Weekends/days off: _________________

3. What is the earliest time you would get up in a normal week?
   
   During the week: ___________________  Weekends/days off: _________________

4. What is the latest time you would get up in a normal week?
   
   During the week: ___________________  Weekends/days off: _________________

5. At what time do you normally have your last meal of the day?
   (i.e. dinner, after-dinner snack)
   
   _____________________

6. How many days per week do you eat breakfast?
   
   _____________________

7. What time do you normally eat breakfast?
   
   During the week: _________________  Weekends/days off: _________________

8. What do you usually eat for breakfast?
8.4 Food Acceptability and Frequency Questionnaire

Experiment 1

1. Please indicate with a rating between 1 and 5 how much you like the following foods (1 = dislike very much; 2 = dislike moderately; 3 = neither like nor dislike; 4 = like moderately; 5 = like very much) and how often you eat them (daily, weekly, monthly, never). If you have never eaten a food before, please indicate this with an X.

<table>
<thead>
<tr>
<th>Enjoyment</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td>Soy Milk</td>
<td></td>
</tr>
<tr>
<td>Almond Milk</td>
<td></td>
</tr>
<tr>
<td>Yogurt Drink (e.g. Yop)</td>
<td></td>
</tr>
<tr>
<td>Cereal</td>
<td></td>
</tr>
<tr>
<td>Cheerios</td>
<td></td>
</tr>
<tr>
<td>Pizza</td>
<td></td>
</tr>
</tbody>
</table>

2. Please rank these pizzas in order of preference from 1 to 3 with 1 = favourite and 3 = least favourite. If there is a pizza you will not eat, please indicate that with an “X”.

<table>
<thead>
<tr>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
</tr>
<tr>
<td>Pepperoni</td>
</tr>
<tr>
<td>Deluxe (pepperoni, red and green bell peppers, onions)</td>
</tr>
</tbody>
</table>

3. If you have never eaten one of the above foods before, would you be willing to eat it during the study? YES □ NO □
Experiment 2

1. Please indicate with a rating between 1 and 5 how much you like the following foods (1 = dislike very much; 2 = dislike moderately; 3 = neither like nor dislike; 4 = like moderately; 5 = like very much) and how often you eat them (daily, weekly, monthly, never). If you have never eaten a food before, please indicate this with an X.

<table>
<thead>
<tr>
<th>Food</th>
<th>Enjoyment</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular Yogurt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greek Yogurt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Jam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. If you have never eaten one of the above foods before, would you be willing to eat it during the study?  
   YES □  NO □
8.5 Eating Habits Questionnaire

Please choose the appropriate answer to best describe your personal situation (last 6 months).

1. How often are you dieting?
   never □ rarely □ sometimes □ often □ always □

2. What is the maximum amount of weight (in pounds) that you have ever lost within one (1) month?
   1 – 4 □ 5 – 9 □ 10 – 14 □ 15 – 19 □ 20 + □

3. What is your maximum weight gain within one (1) week?
   0 – 1 □ 1.1 – 2 □ 2.1 – 3 □ 3.1 – 5 □ 5.1 + □

4. In a typical week, how much does your weight fluctuate?
   0 – 1 □ 1.1 – 2 □ 2.1 – 3 □ 3.1 – 5 □ 5.1 + □

5. Would a weight fluctuation of 5 lbs. affect the way you live your life?
   not at all □ slightly □ moderately □ very much □

6. Do you eat sensibly in front of others and splurge alone?
   never □ rarely □ often □ always □

7. Do you give too much time and thought to food?
   never □ rarely □ often □ always □

8. Do you have feelings of guilt after overeating?
   never □ rarely □ often □ always □

9. How conscious are you of what you are eating?
   not at all □ slightly □ moderately □ very much □

10. How many pounds over your desirable weight were you at your maximum weight?
    0 – 1 □ 2 – 5 □ 6 – 10 □ 11 – 20 □ 21 + □
8.6 Participant Eligibility Summary

Experiment 1

Screening ID: ________  Initials: ________  Age: ________  Gender: ________

Anthropometric Measurements

<table>
<thead>
<tr>
<th>Height (cm):</th>
<th>Weight (kg):</th>
<th>BMI (kg/m$^2$):</th>
</tr>
</thead>
</table>

Eating Habits Questionnaire Score:

Eligibility Checklist:

<table>
<thead>
<tr>
<th>Healthy (no medical conditions)</th>
<th>□ YES  □ NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokes</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>20-30 years old</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>BMI 20.0-24.9 kg/m$^2$ (+ 5 lbs weight fluctuation)</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Uses protein powders/supplements</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Uses prescription medication</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Elite or training athlete</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Trying to lose or gain weight</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Vegan</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Excludes dairy products</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Regular breakfast consumer</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Likes all study foods</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Score of &gt; 11 on Eating Habits Questionnaire</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td><strong>Females Only</strong></td>
<td></td>
</tr>
<tr>
<td>Pregnant or planning on becoming pregnant</td>
<td>□ YES  □ NO</td>
</tr>
<tr>
<td>Regular monthly menstrual cycles</td>
<td>□ YES  □ NO</td>
</tr>
</tbody>
</table>

Is the individual eligible to participate in the study? □ YES  □ NO

Assigned Participant ID#: ____________

Preferred day(s): MON □  TUES □  WED □  THURS □  FRI □  SAT □  SUN □

Preferred time: ____________

Pizza preference (in order): 1 ____________

2 ____________

3 ____________
Experiment 2

Screening ID: ________  Initials: ________  Age: ________  Gender: ________

Anthropometric Measurements

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<tr>
<th>Height (cm):</th>
<th>Weight (kg):</th>
<th>BMI (kg/m²):</th>
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</thead>
</table>

Eating Habits Questionnaire Score:

Eligibility Checklist:

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<tr>
<th>Generally healthy (no medical conditions)</th>
<th>YES</th>
<th>NO</th>
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</thead>
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<td>NO</td>
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<tr>
<td>BMI 18.5-29.0 kg/m²</td>
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<td>NO</td>
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<tr>
<td>No prescription medication affecting appetite and metabolism</td>
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<td>NO</td>
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<td>Trying to lose or gain weight</td>
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<td>NO</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>YES</td>
<td>NO</td>
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<tr>
<td>Excludes dairy products</td>
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<td>NO</td>
</tr>
<tr>
<td>Regular breakfast consumer</td>
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<td>NO</td>
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<tr>
<td>Likes all study foods</td>
<td>YES</td>
<td>NO</td>
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<tr>
<td>Score of &gt; 11 on Eating Habits Questionnaire</td>
<td>YES</td>
<td>NO</td>
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<tr>
<td><strong>Females Only</strong></td>
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<td></td>
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<tr>
<td>Menopausal</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

Is the individual eligible to participate in the study?  □ YES  □ NO

Assigned Participant ID#: ____________

Preferred day(s): MON □  TUES □  WED □  THURS □  FRI □  SAT □  SUN □

Preferred time: ______________
Appendix 9. Study Day Forms

Sleep Habits and Stress Factors Questionnaire
Recent Food Intake and Activity Questionnaire
Motivation to Eat VAS
Physical Comfort VAS
Energy and Fatigue VAS
Treatment and Test Meal Palatability
9.1 Sleep Habits and Stress Factors Questionnaire

1. Did you have a normal night’s sleep last night?
   - YES  - NO

2. How many hours of sleep did you have?
   __________________________________________

3. At what time did you go to bed?
   __________________________________________

4. At what time did you wake up this morning?
   __________________________________________

5. Recount your activities since waking up:
   
<table>
<thead>
<tr>
<th>TIME</th>
<th>ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
</tbody>
</table>

6. Are you experiencing any feelings of illness or discomfort other than those from hunger?
   
   | Today: | YES  - NO |
   | Past 24 hours: | YES  - NO |
   
   If yes, please describe briefly:
   __________________________________________

7. Are you under any unusual stress? (i.e. work deadlines, personal)
   
   | Today: | YES  - NO |
   | Past 24 hours: | YES  - NO |
   
   If yes, please describe briefly:
   __________________________________________

8. Have you been involved in any physical activity, unusual to your normal routine within the past 24 hours?
   - YES  - NO

9. Have you had anything to eat or drink other than water for the past 12 hours?
   - YES  - NO

10. Are there any other circumstances that may affect your participation in the study today?
    - YES  - NO

    If yes, please describe briefly:
    __________________________________________
9.2 Recent Food Intake and Activity Questionnaire

At what time did you have dinner? _____________________

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):  
____________________________________________________________________________________  
____________________________________________________________________________________  
____________________________________________________________________________________  

The following three questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small “ | ” across the horizontal line at the point which best reflects your present feelings.

How would you describe your **food intake** over the past 24 hours?  
| Much LESS than usual | | | Much MORE than usual |
|----------------------|----------------|----------------------|

How would you describe your **level of activity** over the last 24 hours?  
| Much LESS than usual | | | Much MORE than usual |
|----------------------|----------------|----------------------|

How would you describe your **level of stress** over the last 24 hours?  
| Much LESS than usual | | | Much MORE than usual |
|----------------------|----------------|----------------------|
9.3 Motivation to Eat VAS

These questions relate to your “motivation to eat” at this time. Please rate yourself by drawing a vertical line “|” across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

   VERY weak .................................................. | VERY strong

2. **How hungry do you feel?**

   NOT hungry at all ................................ | As hungry as I have ever felt

3. **How full do you feel?**

   NOT full at all ......................................... | VERY full

4. **How much food do you think you could eat?**

   NOTHING at all ........................................... | A LARGE amount

5. **How thirsty do you feel?**

   NOT thirsty at all ...................................... | As thirsty as I have ever felt

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138
9.4 Physical Comfort VAS

These questions relate to your “motivation to eat” at this time. Please rate yourself by drawing a vertical line “|” across the horizontal line at the point which best reflects your present feelings.

1. Do you feel nauseous?

NOT at all | | VERY much

2. Does your stomach hurt?

NOT at all | | VERY much

3. How well do you feel?

NOT well at all | | VERY well

4. Do you feel like you have gas?

NOT at all | | VERY much

5. Do you feel like you have diarrhea?

NOT at all | | VERY much
9.5 Energy and Fatigue VAS

These questions relate to your energy level and fatigue at this time. Please rate yourself by drawing a vertical line “|” across the horizontal line at the point which best reflects your present feelings.

1. **How energetic do you feel right now?**

   | NOT at all | VERY energetic |

2. **How tired do you feel right now?**

   | NOT at all | VERY tired |
9.6 Treatment and Test Meal Palatability

This question relates to the palatability of the meal you just consumed. Please rate yourself by drawing a vertical line “|” across the horizontal line at the point which best reflects your present feelings.

1. How pleasant did you find the treatment/meal?

| NOT at all pleasant | | VERY pleasant |

2. How tasty did you find the treatment/meal?

| NOT at all tasty | | VERY tasty |

3. How did you like the texture of the treatment/meal?

| NOT at all much | | VERY much |

4. How pleasant did you find the overall breakfast/lunch?

| NOT at all pleasant | | VERY pleasant |
## Appendix 10. Blood Glucose and Insulin Tracking Sheet

### Experiment 1

<table>
<thead>
<tr>
<th>Notes</th>
<th>Time (mins)</th>
<th>Time on the Timer</th>
<th>Blood Glucose (mmol/L)</th>
<th>Researcher Initials</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0:00</td>
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<td></td>
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<tr>
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<td>0:15</td>
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<tr>
<td>insulin</td>
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## Experiment 2

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### Appendix 11. Lunch Meal and Water Intake Tracking Sheet

#### Experiment 1

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<th>Researcher</th>
<th>Date</th>
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</table>

<table>
<thead>
<tr>
<th>Tray 1</th>
<th>Amount</th>
<th>Before (g)</th>
<th>After (g)</th>
</tr>
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<tbody>
<tr>
<td>Deluxe</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pepperoni</td>
<td></td>
<td></td>
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</tr>
<tr>
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<table>
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<table>
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<tr>
<th>Session #</th>
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# Experiment 2

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**Notes:**

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