The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

By

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Current evidence suggests potential benefits of foods with high fibre or high protein content on blood glucose control. However, studies comparing the effects of fermented dairy products to commercially available non-dairy alternatives are limited. This study compared the effects of dairy and non-dairy cultured products on postprandial blood glucose, insulin response, subjective appetite, and food intake in young women. Methods: In a randomized single-blinded cross-over design, 24 women (22.7 ± 2.5 yrs; 22.1 ± 1.5 kg/m²) consumed, to satiation, one of three treatments including: (1) Greek yogurt with granola (150kcal, 9.2g protein, 2.6g fat, 2.0g dietary fibre, and 21.5g available carbohydrate/100g), (2) a cultured coconut product with granola (146kcal, 3.2g protein, 3.2g fat, 5.6g dietary fibre, and 21.9g available carbohydrate/100g), or (3) water control. Serum blood concentrations of glucose and insulin were measured immediately before consumption of treatments and for the following 2 hours. Subjective appetite scores were measured immediately before consumption of treatments and for the following 2 hours. Results: Blood glucose was lower after the dairy treatment (P<0.0001) while overall insulin response was higher (P<0.0001) compared to the non-dairy treatment over two hours. No differences in food intake were observed between the caloric treatments consumed to satiation and 2 hours later with a pizza meal, however both treatments resulted in reduced food intake with the pizza meal compared to the control (P<0.003). Subjective appetite was suppressed after the caloric treatments compared to the control (P<0.0001). Treatments formulated with dairy and non-dairy fermented products did not differ in pleasantness, taste, and texture. The reduced blood glucose after the dairy treatment can be explained by its higher protein content compared to the non-dairy treatment which had a high fibre and low protein content. Conclusion: The breakfast meal formulated with the dairy fermented product resulted in reduced postprandial glycaemia without an increase in subsequent energy intake and can be recommended as a functional breakfast for improved blood glucose control.
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Chapter 1: Introduction

Worldwide overweight and obesity rates are on the rise, with an estimated 1.9 billion overweight adults in 2014 and, of them, 600 million obese individuals (World Health Organization 2015). Overweight and obesity are associated with increased risk of a variety of comorbidities including cardiovascular disease, type 2 diabetes, and some cancers (World Health Organization 2015). In Canada, as around the world, the upward trend of overweight, obesity, and associated diseases is concerning from both a public health and economic perspective (Public Health Agency of Canada 2011, Statistics Canada 2015). In particular, type 2 diabetes is associated with high health care costs, with an estimated increase in healthcare costs of three to four times in a diabetic population as compared to a nondiabetic population (Public Health Agency of Canada 2011). It is imperative, with an aging population in Canada, to work towards reducing rates of overweight and obesity through a variety of avenues, including dietary and lifestyle factors. One such area to be explored is the potential that lies within food consumption. Dietary interventions are a significantly lower cost option compared to pharmaceuticals and surgical interventions as potential weight loss strategies (Encinosa, Bernard et al. 2005). It is well documented that breakfast skipping is a prevalent habit in adolescents and adults that is also associated with weight gain (Forslund, Torgerson et al. 2005, Song, Chun et al. 2005, Niemeier, Raynor et al. 2006, Keski-Rahkonen, Bulik et al. 2007). Thus, breakfast is an ideal meal to target to potentially influence adult body weight. Eating habits are known to differ between men and women and differences in the hormonal responses to food as well as responses to sensory characteristics of foods have been observed (Carroll, Kaiser et al. 2007, McCrickerd, Chambers et al. 2014). By investigating foods that can be consumed for
breakfast that also offer additional benefits in terms of satiety and blood glucose control, there is the potential to have a multi-faceted influence on individuals through the food consumed for breakfast. One such common breakfast food is yogurt which is recommended, low fat varieties, by the Canadian Diabetes Association as a healthy food item to help with blood glucose control (Canadian Diabetes Association, 2013). A crossover study comparing high (24.9g protein/160kcal), moderate (14g protein/160kcal), and low (5g protein/160kcal) protein yogurts provided as an afternoon snack observed a delay in the request for a subsequent meal, higher ratings of fullness, and reduced feelings of hunger in women after the high protein yogurt snack compared to the moderate and low protein yogurt (Douglas, Ortinau et al. 2013), Another crossover study in women also investigated high protein (14g protein/160kcal) compared to normal protein (5g protein/160kcal) in yogurt snacks, however the researchers did not find significant differences in perceived hunger or fullness or ad libitum subsequent meal intake (Ortinau, Culp et al. 2013). In another study, plain yogurt was seen to result in reduced 30 minute post prandial blood insulin peaks as compared to other snacks (honey sweetened yogurt, strawberry yogurt, and orange juice) and also showed a significantly lower blood glucose response as compared to the orange juice treatment (Khoury, Brown et al 2014). Given the sex-related differences in food consumption and various responses to food, investigating the effects of foods on men and women separately in an experimental setting is warranted.

This project aims to investigate the relationship between the consumption of dairy and non-dairy fermented products consumed with breakfast cereal and glycaemic control, short-term food intake, and satiety in young women.
Chapter 2: Literature Review

1) Metabolic Disorders in Canada

a) **Obesity:**

Obesity rates are on the rise in North America and around the world and along with them comorbidities including diabetes and cardiovascular disease (Public Health Agency of Canada 2011). WHO estimates place 2014 worldwide rates of overweight and obesity at 1.9 billion adults overweight and 600 million obese (World Health Organization 2015). Obesity is also a significant risk factor in the development of metabolic syndrome, which is the presence of multiple risk factors like high blood pressure, high cholesterol, excess abdominal fat, and hyperglycaemia, that increase the risk of atherosclerosis and diabetes (Furukawa, Fujita et al. 2004). The accumulation of fat associated with obesity has been seen to increase the rate of oxidative stress in both humans and rats due to the production of adipokines (Furukawa, Fujita et al. 2004, Fernandez-Sanchez, Madrigal-Santillan et al. 2011). Adipokine activity, particularly dysregulation of adipokine production where adipokines like leptin and tumor necrosis factor are overproduced and others such as Adiponectin are under produced, may then lead to the diseased states of diabetes and atherosclerosis through impaired glucose uptake, reduced secretion of insulin, and oxidative stress on vascular cell walls (Furukawa, Fujita et al. 2004).

Excess weight increases the risk of a variety of diseases including cardiovascular disease, type 2 diabetes, and some forms of musculoskeletal disorders (ie. Osteoarthritis) and cancers (World Health Organization 2015). Likewise, rates and the associated economic and healthcare burden
of comorbidities are also on the rise (Public Health Agency of Canada 2011). The causes of overweight and obesity are complex and not fully understood, however a wide variety of factors are thought to play a role including psychological, socioeconomic, genetic, and environmental factors (Naukkarinen J 2012). Some of these factors are very difficult to influence on an individual basis, however the environmental influence of dietary intake and physical activity can be affected, thus making them important factors in the mitigation of overweight and obesity.

b) Type 2 Diabetes:

Diabetes is a disease where an individual’s body doesn’t produce sufficient insulin or is unable to properly utilize the insulin that is produced. Type 2 diabetes is where the body doesn’t properly use the insulin produced due to insulin resistance or doesn’t produce enough insulin (Canadian Diabetes Association 2012). Rising diabetes rates in Canada poses a significant public health concern from both a social and economic perspective due to the increased health care needs associated with diabetes (Public Health Agency of Canada 2011). Individuals with diabetes visit health care professionals, including their primary physician, specialists, and experience hospitalization, more frequently than individuals without diabetes which results in an estimated increase in health care costs by three to four times compared to a non-diabetic population (Public Health Agency of Canada 2011). The healthcare system cost of diabetes in 2010 was $11.7 billion and is projected to continue to rise (Canadian Diabetes Association 2012). It is predicted that a mere 2% decrease in the prevalence of diabetes in Canada would result in a reduction of 9% in healthcare costs directly associated with the disease, therefore
strategies to reduce diabetes prevalence and the severity of disease have the potential to have a significant economic benefit (Canadian Diabetes Association 2012).

2) Dietary Factors

a) Role of Food and Eating Factors in Metabolic Disorders:

The increased consumption of energy dense foods, particularly convenience foods, in conjunction with low consumption of nutrient dense foods is well documented as an environmental contributing factor to the high rates of overweight and obesity in North America (Bowman, Gortmaker et al. 2004, Bowman and Vinyard 2004, Rosenheck 2008). The consumption of high sugar foods, particularly sugar sweetened beverages, has been somewhat controversially linked to weight gain, obesity, and the development of type 2 diabetes (Bray, Nielsen et al. 2004, Stanhope 2012). Despite this, observational research has linked high sugar consumption to increased risk of type 2 diabetes (Sonestedt, Overby et al. 2012) and large health organizations like the World Health Organization and the Heart and Stroke Foundation have made recommendations pertaining to the intake of free sugars (Foundation 2014, World Health Organization 2015). Eating habits such as skipping breakfast and frequent snacking have also been indicated as contributors to weight gain in both adolescents and adults and have been noted to differ between obese and normal weight individuals (Forslund, Torgerson et al. 2005, Song, Chun et al. 2005, Niemeier, Raynor et al. 2006, Keski-Rahkonen, Bulik et al. 2007).
b) **Restrained eating habits:**

Restrained eating refers to behaviours that some individuals employ in an effort to avoid gaining weight (van Strien, Frijters et al. 1986). Several instruments have been developed that measure restrained eating, including the Dutch Eating Behaviour Questionnaire (van Strien, Frijters et al. 1986), Three-Factor Eating Questionnaire (Stunkard and Messick 1985), and the Restraint Scale (Herman and Mack 1975). Findings from studies using eating restraint scales have found differences in the quantity of food consumed by healthy weight no restrained vs. restrained eaters, with no restrained eaters eating less food after a preload than restrained eaters (van Strien, Frijters et al. 1986). However, this difference was reversed in restrained obese individuals in a separate study (Ruderman and Christensen 1983). Thus, when studying food consumption it is important to consider individual eating restraint behaviours and BMI as factors.

The three factor eating questionnaire (TFEQ), now called the Eating Inventory (EI), was developed in the late 1980’s to expand eating restraint scales to obese individuals (Westenhoefer, Stunkard et al. 1999). It consists of 51 questions that measure three eating behaviour factors: cognitive restraint, disinhibition, and hunger (Stunkard and Messick 1985). It was later observed that restrained eaters were not a uniform group and the questionnaire was further improved upon by dividing cognitive restraint into two subscales: rigid control and flexible control (Westenhoefer, Stunkard et al. 1999, Johnson, Pratt et al. 2012).

A study investigating the identification of relationships between the dietary restraint subscales found that in both men and women, high scores on flexible control correlate with weight loss.
success, lower BMI, and reduced energy intake, whereas high scores on rigid control correlate with higher BMI, increased energy intake, and reduced weight loss success in women only (Westenhoefer 1991). Another study also linked rigid dietary control to reduced weight loss success and flexible dietary control to increased weight loss success with food cravings observed as a mediator between rigid control and dieting but not for flexible control (Meule, Westenhoefer et al. 2011). These relationships between scale scores have been observed over large samples of men and women and have been deemed to be valid predictors of behaviour, particularly in using flexible control as a BMI predictor (Williamson, Lawson et al. 1995, Westenhoefer, Stunkard et al. 1999). Additional studies differ in their findings with regards to correlations between weight loss and rigid or flexible control, particularly when stratified by gender (Burgmer, Grigutsch et al. 2005, Timko and Perone 2005).
3) Regulation of Short-Term Food Intake

a) Satiety and Satiation:

Satiety is referred to as the cessation of motivation to eat, a decrease in an individual’s level of hunger, and a state of fullness after the consumption of food (Bellisle, Drewnowski et al. 2012, Rebello, Johnson et al. 2013) and affects the amount of time that elapses between episodes of eating (Aziz and Anderson 2007). Satiety is influenced by a variety of factors, such as the energy and nutrient content of a food, and physical form of the food, related to the consumption of a food and the food being consumed. Research continues to improve the understanding of the interactions between food components, the eating environment, psychological factors, digestion, and satiety and satiation. In studies on satiety and satiation, it is important to replicate real-life eating behaviours in that foods are consumed *ad libitum* in experiments as they would be in typical eating situations (Green, Delargy et al. 1997).

A number of methods have been used scientifically to assess satiety including the use of scales to measure subjective feelings (Merrill, Kramer et al. 2002). The use of visual analog scales is the most common method of assessing satiety through questions on motivation to eat, prospective food consumption, hunger, fullness, and satiety (Chapelot 2013). Another tool used is the satiety cascade which looks at the effect of foods on both satiation and satiety and suggests that food composition, caloric density, physical and sensory properties, postingestive and postabsorptive processes all have an effect on both satiety and satiation (Blundell, Green et al. 1994, Green, Delargy et al. 1997, Blundell, De Graaf et al. 2010). These effects are easier to observe in a research setting as the satiety cascade may also be impacted by factors such as the
environmental context in which the food is consumed, environmental cues like portion size, as well as learned satiation responses to common foods (Blundell, De Graaf et al. 2010). Satiety has also been objectively evaluated using the timing of meal requests to assess satiety, however this method requires that participants are effectively time blinded to prevent typical meal timing conditioning to affect the participants behaviour (Chapelot 2013).

Satiation refers to the bodily mechanisms, stretch receptors in the stomach and hormonal responses, that result in the termination of an eating episode (Aziz and Anderson 2007). Meal cessation has been stated by study participants to be primarily due to either fullness or boredom of taste (Rolls, Rolls et al. 1981, Poothullil 1995, Blundell, De Graaf et al. 2010). Three sensory mechanisms are thought to be involved in satiation: conditioned satiety, alimentary alliesthesia, and sensory-specific satiety which all act by reducing the pleasantness of food stimuli (Brondel, Romer et al. 2007). The physical characteristics of a food product, particularly dairy products, have an effect on individual perceptions of its satiating capacity which also plays into conditioned satiety (Hogenkamp, Stafleu et al. 2011). It has been found that texture plays a significant role in expected satiation, with increased thickness of a dairy product being associated with increase expected satiation of the product in one study which would likely be a result of conditioned satiety (Hogenkamp, Stafleu et al. 2011). Oral satiation may also play a role, which relates to the nutrient and energy content of the food being consumed and its effect on termination of consumption (Poothullil 1995). Metabolic factors also play a role in the quantity of food consumed and include gastrointestinal signals to the brain indicating stomach
fullness (stretch receptors) and hunger/satiety hormones (ghrelin, cholecystokinin, GLP-1, PYY) (Blundell, De Graaf et al. 2010). An experiment comparing appetite after soup consumption via the mouth, direct infusion into the stomach, and direct infusion into the small intestine confirms a role of gastric stimulation in hunger suppression (Cecil, Francis et al. 1998). This study found the greatest appetite suppression in the condition where soup was consumed orally, supporting the theory of a relationship between gastric emptying and orosensory mechanisms (Cecil, Francis et al. 1998). Thus, in studying satiation, the palatability, energy density, and state (liquid vs. solid) of the foods consumed must be considered along with the participant’s state of satiety, time until their next meal, and potentially learned responses about specific foods (Blundell, De Graaf et al. 2010).

Consumer deemed palatability of a food has a positive influence on the quantity of food eaten in a wide variety of food products, over different hunger states, and in both obese and non-obese individuals of both genders (De Graaf, De Jong et al. 1999). The pleasantness of a food is thought to affect consumer satiation levels with more pleasant foods associated with higher ad libitum food intake and less pleasant foods with lower ad libitum intake (De Graaf, De Jong et al. 1999). Food preference also appears to play a role in desire to eat with the availability of a preferred food observed to increase the desire to eat in female participants (Hill, Magson et al. 1984). However, further confounding the matter, the pleasantness of a food has also been noted to be related to the existing satiety status of individuals with foods being rated more pleasant by fasted vs. satiated individuals (Poothullil 1995). Study results have come to varying conclusions with regards to the effect of pleasantness of a food on post meal satiety. Some studies have found no difference in post meal hunger ratings between more and less liked
meals (De Graaf, De Jong et al. 1999) whereas others have found increased hunger ratings after consumption of more pleasant meals (Hill, Magson et al. 1984, De Graaf, De Jong et al. 1999).

b) Effect of macronutrients on satiety and food intake:

Experiments using satiety quotients have confirmed that the nutrient composition of a food also has an effect on its ability to satiate (Green, Delargy et al. 1997). They further demonstrate the complexity of the relationships between food intake, satiety, and satiation. Research on the macronutrients suggests a hierarchy of effect on short term satiety with protein having the greatest ability to satiate and fat the least (Blundell and MacDiarmid 1997, Green, Delargy et al. 1997, Paddon-Jones, Westman et al. 2008, Veldhorst, Smeets et al. 2008). However, the effect of fat and carbohydrates on satiety is not as clear cut as protein with time post ingestion appearing to also play a role (Green, Delargy et al. 1997). In one experiment, a low energy high-carbohydrate preload was found to have the greatest immediate satiating effect compared to a high energy high-carbohydrate and high energy high fat preload, however it was found to be the least satiating preload at two hours post consumption (Green, Delargy et al. 1997). The high energy high carbohydrate preload was the most satiating at two hours post consumption (Green, Delargy et al. 1997). In a similar experiment, it was observed that a set quantity of two low energy preloads (one sweetened with sucrose, the other sweetened with aspartame) both initially had a greater hunger reducing effect, however were less satiating than the two high energy preloads also sweetened with sucrose and aspartame one hour after consumption (Green, Delargy et al. 1997). A different experiment found a similar satiety effect of high fat versus carbohydrate preload drinks but observed that the satiating effect of the fat preload
lasted longer than that of the carbohydrate preload (Melanson, Westerterp-Plantenga et al. 1999). When comparing a high carbohydrate and a high fat yogurt with similar protein content, no difference was observed in male or female participants hunger or fullness ratings between the treatments (Rolls, Kim et al. 1991). An experiment on ad libitum consumption found that per kJ of intake, high fat foods initially reduced hunger less than high carbohydrate foods but by one hour post consumption there was no difference between high fat and high carbohydrate foods in terms of satiety (Green, Delargy et al. 1997). Given the lower initial satiating effect of high fat foods, combined with the results seen in the ad libitum consumption of participants in this study where more energy was consumed when high fat foods were provided, it is posited that individuals consume greater quantities of high fat foods due to their reduced ability to satiate short term. That, combined with the greater number of calories per gram contained in fat, suggests a concerning possibility of caloric overconsumption of high fat foods, which is a nutritional attribute of many of the convenience foods available today.

Vandewater and Vickers found in one study (Vandewater and Vickers 1996) that two different high protein meals (73g protein/463g treatment and 39g protein/265g treatment) resulted in greater satiety compared to low protein versions (34g protein/463g treatment and 12g protein/182g treatment) of the same meals. Protein appears to increase satiety post-meal both in short term and long term studies, with long-term studies seeing a continuous effect of increased satiety over the day (van Meijl, Vrolix et al. 2008, Veldhorst, Smeets et al. 2008). However, the effect of protein on satiety also appears to be influenced by the type of protein consumed (Paddon-Jones, Westman et al. 2008, van Meijl, Vrolix et al. 2008, Veldhorst, Smeets et al. 2008). Whey and casein proteins, in particular, appear to have unique effects on satiety
Additionally, protein has been observed to have an increased satiating effect only when consumed in excess of individual protein requirements (Lejeune, Westerterp et al. 2006, Veldhorst, Smeets et al. 2008).

c) **Role of sex:**

It is well known that eating behaviours differ between men and women. Men consume, on average, more food than women due to their higher resting metabolic rate and, typically, higher weight (Rolls, Fedoroff et al. 1991). Sex differences in postprandial hormone responses have also been observed (Carroll, Kaiser et al. 2007). Through brain imaging, marked differences between men and women have been seen in brain responses to hunger and satiation in terms of processing the cognitive and emotional aspects of those eating stimuli (Del Parigi, Chen et al. 2002). In a study looking at the effect of thickness and creaminess of a beverage on intake it was observed that female participants were affected by the thickness of the beverage and subsequently consumed less whereas male participant consumption was not affected by either thickness or creaminess (McCrickerd, Chambers et al. 2014). Despite the difference in consumption, there was no difference between men and women in terms of ratings of hunger, fullness, or desire to eat (McCrickerd, Chambers et al. 2014). However, in a male only study comparing differences in thickness and creaminess of high protein (32-33g protein/300g) beverages and a thick and creamy high carbohydrate (58g carbohydrate/300g) beverage found a significant reduction in intake of the thick and creamy high protein and high carbohydrate beverages as compared to the thin high protein beverage suggesting an effect of sensory characteristics on short-term intake in men (Bertenshaw, Lluch et al. 2013). Thus,
while the sex specific differences are still not fully understood in terms of food consumption and responses, it is quite clear that they do exist and should be accounted for in study designs. Furthermore, differences in food intake in young women during the phases of the menstrual cycle have been observed with fluctuations in estradiol concentrations posited as a potential reason for the difference (Alonso-Alonso, Ziemke et al. 2011). It has been observed that food intake is reduced in the periovulatory phase and significantly increased in the luteal phase compared to the follicular phase (Buffenstein, Poppitt et al. 1995, Pelkman, Heinbach et al. 2000). However, these differences are thought to only been seen when ovulation occurs; individuals who experience an anovulatory cycle don’t appear to have a significant difference in caloric intake over the cycle (Buffenstein, Poppitt et al. 1995). Restrained eaters, on the other hand, appear to differ in their cyclic response to food intake, with no significant increase in food consumption observed during the luteal phase (Schweiger, Tuschl et al. 1992). Additionally, glucose metabolism has been linked to estrogen levels and insulin sensitivity to estradiol, progesterone, and follicle stimulating hormone levels, all of which fluctuate during the menstrual cycle (Schisterman, Mumford et al. 2014). An association between blood glucose and menstrual cycle phases has not, however, been consistently repeated in other studies (Buffenstein, Poppitt et al. 1995). Studies looking at oral contraceptive use have observed no significant difference in food intake as compared to non-users (Tucci, Murphy et al. 2010) as well as increased food intake in oral contraceptive users (Wallace, Heiss et al. 1987, Eck, Bennett et al. 1997). Newer oral contraceptives contain lower levels of hormones and are touted to have a reduced effect on blood glucose and insulin levels and no effect of increasing
weight or blood pressure (Cerel-Suhl and Yeager 1999), therefore the effects seen in older studies may be lessened with the prevalence of newer, lower hormone, oral contraceptive use.

Thus to minimize the effect of female reproductive hormones on relevant study factors, it is important to measure participants at the same phase of their menstrual cycle. Similarly, given that there are known gender differences in eating behaviours (Del Parigi, Chen et al. 2002), the observed differences in processing some hunger and satiety signals suggest that there is value in separating satiety study participants by gender.
4) Regulation of Postprandial Glycaemia

Glycemic control is an important aspect of diabetes management in terms of reducing the incidence and severity of diabetic complications. Given that elevated postprandial blood glucose levels is a risk factor associated with type 2 diabetes (American Diabetes Association 2001, Brand-Miller, Holt et al. 2002), an understanding of the effect of various foods on blood glucose levels is becoming increasingly relevant from a health standpoint.

A number of studies have demonstrated a link between post meal blood glucose response and feelings of hunger (Campfield, Smith et al. 1996, Himaya, Fantino et al. 1997, Gielkens, Verkijk et al. 1998, Melanson, Westerterp-Plantenga et al. 1999). It has been observed that a decline in blood glucose occurs prior to meal consumption initiation (Campfield, Smith et al. 1996) and that insulin induced hypoglycaemia can result in an increase in hunger ratings and subsequent food intake in men (Dewan, Gillett et al. 2004). In a study that compared rapid vs. slow consumption of a glucose beverage in conjunction with a set meal schedule in obese and overweight women, the researchers found statistically significant interactions between glucose condition, time, and appetite scores (Arumugam, Lee et al. 2008). Blood insulin and glucose levels were able to be used to significantly predict fullness, hunger, appetite score, and prospective consumption (Arumugam, Lee et al. 2008). Meal requests from time blinded individuals in one study corresponded with a post preload blood glucose decline (Melanson, Westerterp-Plantenga et al. 1999). This relationship between hunger and blood glucose levels has also been observed in other studies (Campfield, Smith et al. 1996, Himaya, Fantino et al. 1997, Gielkens, Verkijk et al. 1998). It has been posited that a drop in blood glucose levels over
a period of time signals the desire to eat rather than reaching a specific blood glucose concentration (Melanson, Westerterp-Plantenga et al. 1999). In one study, it was observed that a drop of blood glucose levels to 8% below baseline preceded a meal request in time-blind individuals (Campfield, Smith et al. 1992) and it was noted that if food was not provided before blood glucose levels return to baseline, a spontaneous meal request didn’t occur until after an additional drop in blood glucose levels occurred (Campfield and Smith 1986, Himaya, Fantino et al. 1997).

Foods that produce a lower blood glucose response have been found to be more satiating than those that produce a higher blood glucose response which is thought to be due to the slower digestion and absorption rate of these foods (Brand-Miller, Holt et al. 2002). There may also be an interaction between blood glucose levels and dairy as post-meal blood glucose response has been shown to be significantly lower after a dairy-containing meal as compared to a comparable dairy-free meal, however that response was only seen in low fat meals (Schneeman, Burton-Freeman et al. 2003).

a) **Effect of dietary proteins on blood glucose control:**

Blood glucose levels are affected by a variety of factors related to meal consumption, such as the quantity of food consumed, timing of eating occurrence, and the macronutrient composition of the food consumed (American Diabetes Association 2001). Additionally, differences have been observed in the postprandial blood glucose responses in normal weight versus obese men and women, with higher postprandial blood glucose levels seen in obese
individuals as well as an increased period of elevated postprandial blood glucose levels as compared to normal weight individuals (Carroll, Kaiser et al. 2007).

A study comparing the effect of a fat versus simple carbohydrate preload drink found that the blood glucose curve after the carbohydrate drink spiked quickly, with the decline in blood glucose seen in the hour post ingestion, whereas the fat preload resulted in a longer, more gradual curve, with the blood glucose decline seen two hours post ingestion (Melanson, Westerterp-Plantenga et al. 1999). However, the effect of carbohydrate versus fat in terms of satiating capacity is not conclusive, with studies finding conflicting results (Hulshof, De Graaf et al. 1993, Blundell, Burley et al. 1998) and different results in obese individuals (Lawton, Burley et al. 1993). In healthy, nondiabetic, individuals, postprandial blood glucose levels typically peak approximately one hour after meal initiation and fall back to pre-prandial levels two to three hours after meal initiation (American Diabetes Association 2001).

b) Insulin-dependent and insulin-independent mechanisms of blood glucose regulation:

It has been observed that postprandial glycaemia is regulated via both insulin-dependent and insulin-independent mechanisms after the consumption of whole milk (Panahi, El Khoury et al. 2014). It is posited that the insulin-independent effect of whole milk on blood glucose is due to a decrease in the rate of stomach emptying as a result of the actions of cholecystokinin, peptide YY, and glucagon-like peptide-1 which are stimulated by milk components (Havel 2001, Panahi, El Khoury et al. 2014). Whey protein has also been observed to have a reduced postprandial glycemic response as compared to a glucose preload in healthy men (Akhavan, Luhovyy et al.
2012). This is posited to also be linked to the slowed gastric emptying rate associated with milk products in addition to the secretion of insulin (Akhavan, Luhovyy et al. 2012).

The postprandial glycemic response to dairy products is likely related to the protein content of the dairy product in addition to other attributes like texture, the presence of other nutrients, and the interaction between all components. Given that dairy proteins interact with and influence various gut hormones, as seen in the study by Panahi et al. (2014), products with a higher protein content could be anticipated to have a greater effect on postprandial outcomes.

c) Gastrointestinal hormones involved in food intake and glycaemic regulation:

A number of hormones are known to affect food intake and the regulation of the glycemic response, including cholecystokinin, ghrelin, and glucagon-like peptide-1. Research is ongoing to fully understand the mechanisms of these hormones, however the gastrointestinal hormones are primarily known for their short-term effects on food intake. However, insulin, a hormone involved in glycemic regulation, is known to have longer term effects.

Cholecystokinin is a gut hormone involved in digestion as well as appetite regulation (Simpson, Parker et al. 2012). Postprandially, cholecystokinin release is stimulated by the presence of amino acids and fatty acids with a carbon chain of eleven or longer in the chyme (Havel 2001, Simpson, Parker et al. 2012). There are two cholecystokinin receptor proteins: CCK-1R and CCK-2R; CCK-1R receptor proteins are found mainly in the pancreas, gall bladder, stomach, kidney, and lung, and are thought to be primarily involved in satiety and food intake (Simpson, Parker
et al. 2012). The precise mechanism of cholecystokinin activity in relation to food intake is not fully understood, however it appears to involve the vagus nerve and suppressing the expression of several receptors as well as an interaction with leptin (Simpson, Parker et al. 2012). The suppression of food intake as a result of CCK release is thought to be due to the effect CCK has on inhibiting gastric emptying (Havel 2001). Cholecystokinin is released after dairy-containing meal consumption, with greater concentrations seen after consuming higher fat meals, particularly in women (Schneeman, Burton-Freeman et al. 2003). The combination of protein and fat present in dairy may help to explain this as the digestion of protein is required for the release of CCK (Liddle 1995).

Ghrelin is a short-term appetite stimulating hormone that is released from the stomach and is inhibited by food intake (Luhovyy, Akhavan et al. 2007, Hall, Heymsfield et al. 2012, Kirchner, Heppner et al. 2012). In a study on obese individuals and those with anorexia nervosa it was observed that ghrelin secretion changes with the energy balance status of individuals – it is increased in negative energy balance and decreased in positive energy balance, thus it is posited that ghrelin plays a role in energy homeostasis and the regulation of eating behaviours through its effects on satiety (Shiiya, Nakazato et al. 2002). Blood ghrelin concentrations have also been observed to be lower in obese compared to lean individuals (Havel 2001, le Roux, Patterson et al. 2005). Postprandial ghrelin concentrations appear to be suppressed differently in obese vs. normal-weight individuals (le Roux, Patterson et al. 2005). Normal-weight individuals have been observed to have a proportional level of ghrelin suppression to caloric
consumption whereas obese individuals have a lower reduction in ghrelin levels, which is thought to potentially play a role in reduced satiety, contributing to obesity maintenance (le Roux, Patterson et al. 2005). The findings of additional research on ghrelin in starvation, however, are not conclusive with regards to ghrelin increasing during prolonged starvation (Kirchner, Heppner et al. 2012).

The effect of food on ghrelin appears to vary depending on the macronutrient, with meal volume having a limited effect on ghrelin concentrations (Erdmann, Topsch et al. 2004, Luhovyy, Akhavan et al. 2007). Carbohydrates appear to have a suppressing effect on ghrelin, potentially due to the increase in blood glucose and insulin levels resulting from carbohydrate consumption as both blood glucose and insulin are also ghrelin suppressors (Lejeune, Westerterp et al. 2006). Conversely, protein appears to have a postprandial effect of increasing ghrelin levels (Erdmann, Topsch et al. 2004). However, in a study comparing a high protein and high carbohydrate yogurt breakfast fed to healthy men, it was found that the high protein breakfast resulted in decreased ghrelin secretion compared to the high carbohydrate breakfast with no significant effect on short term appetite measures and ad libitum (Blom, Lluch et al. 2006). Fat is thought to be the least effective macronutrient at suppressing ghrelin (Kirchner, Heppner et al. 2012).

Glucagon-like Peptide-1 (GLP-1) is a gut hormone produced in the small intestine that is stimulated by the consumption of carbohydrate and fat (Havel 2001, Akhavan, Panahi et al. 2009). GLP-1 stimulates insulin release, inhibits glucagon release, and is involved in the
inhibition of gastric emptying, resulting in food intake suppression (Havel 2001, Akhavan, Panahi et al. 2009, Gallwitz 2012). The effect GLP-1 has on insulin and glucagon is dependent on the presence of glucose (Gallwitz 2012). In mixed meals, protein has been found to have a greater effect on increasing blood GLP-1 concentrations than either carbohydrate or fat (Lejeune, Westerterp et al. 2006). However milk proteins appear to uniquely stimulate the release of GLP-1 regardless of carbohydrate or fat presence (Luhovyy, Akhavan et al. 2007, Akhavan, Panahi et al. 2009) which may help explain the short term satiating effect seen with milk product consumption.
5) Role of Dairy Products and Ingredients in the Regulation of Short-Term Food Intake and Blood Glucose Control

a) Dairy

Significant research has been conducted on the effects of dairy products and dairy proteins on satiety (Panahi, Al Khoury et al. 2013). While research on the effect of dairy products on weight loss is thus far inconclusive (Lanou and Barnard 2008, Chen, Pan et al. 2012), there have been promising findings in terms of the effect on insulin response and satiety (Schneeman, Burton-Freeman et al. 2003). Despite the inconclusiveness of the weight-loss effects of dairy, milk has been linked to lower subjective appetite scores compared to a variety of other common beverages including water, cola, orange, and diet cola, suggesting that dairy has the potential to influence short-term food intake (Panahi, Al Khoury et al. 2013). A meta-analysis of randomized controlled trials looking at dairy intake on body weight and body fat found that in studies where energy restriction was involved, the dairy group experienced a significant reduction in body weight, however in studies without energy restriction, there was no significant weight loss in the dairy group (Chen, Pan et al. 2012). Correspondingly, body fat was significantly reduced in the studies with energy restriction and not in those that didn’t reduce energy (Chen, Pan et al. 2012). However, these results are complicated by the effect of time. The dairy intervention groups presented with marginally significant weight and fat gains in long-term trials that did not restrict energy (Chen, Pan et al. 2012). Another meta-analysis, looking at clinical trials assessing dairy or calcium intake with and without energy restriction on body weight and fat came to similar conclusions (Lanou and Barnard 2008). No significant effect was seen of dairy
consumption on body weight or adiposity in the majority of studies that did not restrict energy, with two of those studies observing weight gain in populations of older adults (Lanou and Barnard 2008). In trials that incorporated energy restriction, the majority found no significant effect of weight loss with dairy or calcium consumption, with three short-term studies, by the same author, observing significant weight loss in the dairy treatment groups (Lanou and Barnard 2008).

b) **Milk Proteins**

The proteins found in milk, specifically whey and casein, are thought to play a role in short-term intake suppression, and the stimulation of satiation and satiety signalling mechanisms (Ebringer, Ferencik et al. 2008, Akhavan, Panahi et al. 2009). Whey and casein proteins have different digestion properties – whey proteins are considered ‘fast’ as they are more soluble and are thus digested quickly whereas casein proteins are considered ‘slow’ as stomach acids cause them to clot and thus digest more slowly (Boirie, Dangin et al. 1997). Whey protein elicits a plasma amino acid increase that peaks 1-2 hours after consumption whereas casein elicits an increase that lasts approximately 7 hours post consumption (Aziz and Anderson 2007). Dairy appears to play a role in the release of various satiety hormones, specifically, cholecystokinin (CCK), ghrelin, and glucagon-like peptide-1 (GLP-1) which is likely linked to the observed short term intake suppression with dairy product consumption (Schneeman, Burton-Freeman et al. 2003, Luhovyy, Akhavan et al. 2007, Akhavan, Panahi et al. 2009, Simpson, Parker et al. 2012).
Milk has been linked to reduced postprandial blood glucose concentrations in a number of experimental studies which is posited to be related to the presence of milk proteins (Panahi, Al Khoury et al. 2013, Panahi, Luhovyy et al. 2013). Milk, both 2% milk fat and 1% milk fat chocolate milk, has been observed to result in a lower postmeal blood glucose concentration after meal consumption as compared to water, soy milk, and infant formula (Panahi, Luhovyy et al. 2013). In this particular study, the lower blood glucose concentration seen with the chocolate milk, despite its higher sugar content, points to some component of milk having a blood glucose lowering effect, likely the ratio and presence of milk proteins (Panahi, Luhovyy et al. 2013).

c) Lactose and milk fat:

While milk proteins are known to play a role in regulating food intake, milk fat has also been shown to help regulate metabolism, in particular through its action on cholecystokinin and peptide YY, stomach emptying rates and the ileal brake (Maljaars, Romeyn et al. 2009). Different types of fat appear to affect satiety differently with the length of the fat chain associated with the degree of hunger and food intake reduction (Feltrin, Little et al. 2004, Maljaars, Romeyn et al. 2009). This is likely due to the stimulation of cholecystokinin release, which inhibits food intake, by longer carbon chain fats (Havel 2001, Feltrin, Little et al. 2004, Simpson, Parker et al. 2012). In one study, unsaturated fatty acids were observed to increased satiety in healthy participants whereas saturated fats appear to have no effect on satiety ratings when the fat was directly infused into the participants ileum (Maljaars, Romeyn et al. 2009). Additionally, lactose, as compared to other sugars, has been seen to result in a lower
glycemic response after consumption of a pure lactose and water solution (Ostman, Liljeberg Elmstahl et al. 2001).

d) Calcium:

Calcium is well known as an important micronutrient for humans, particularly in terms of developing and maintaining bone health (World Health Organization 2005). Calcium ions also play an integral role in many other intracellular body functions (World Health Organization 2005). Research also links calcium to body composition, with lower calcium intakes associated with higher fat content, particularly in women (Jacqmain, Doucet et al. 2003). In one weight reduction trial, the supplementation of calcium and vitamin D was linked to improved fat loss in overweight and obese women who were categorized as low calcium consumers (Major, Alarie et al. 2007, Major, Alarie et al. 2009). The researchers posited a potential appetite controlling effect of calcium as one of the causes of this finding (Major, Alarie et al. 2009). In addition, high calcium intake has been associated with a favourable lipid profile, suggesting a potential benefit in terms of heart disease risk (Jacqmain, Doucet et al. 2003, Major, Alarie et al. 2007).

e) Milk and fermented dairy products:

Yogurt is a milk product that is produced by inoculating homogenized milk with lactic acid bacterial cultures, typically *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and then allowing the milk to ferment (Station 2007, Sun Wang 2009). This fermentation process results in changes in the milk, leading to a reduction in the amount of lactose and an increase in the amount of galactose present in the yogurt as compared to milk (Lourens-Hattingh and Viljoen 2001). After the fermentation process is complete, many commercial products have live
bacterial cultures added to them, such as *L. acidophilus* and *L. bifidus* (Sun Wang 2009). The probiotic content of yogurt has been linked to improved gut function and gut immunity (Adolfsson, Meydani et al. 2004). Dairy is by far the most popular carrier for probiotics, primarily in the form of yogurt (Heller 2001). To glean the benefits of probiotics from a commercial product, the bacteria must be able to survive the processing of the product as well as the human digestion process (Heller 2001, Lourens-Hattingh and Viljoen 2001). There is some debate as to whether *L. acidophilus* and *Streptococcus thermophilus* are able to survive digestion (Lourens-Hattingh and Viljoen 2001), however several experiments have confirmed the passage of those bacteria through the digestive tract (Mater, Bretigny et al. 2005, Elli, Callegari et al. 2006). The bacteria *L. acidophilus* and *B. bifidum* are thought to be more able to survive digestion (Lourens-Hattingh and Viljoen 2001) and are found in many commercial yogurt products. In Canada, products must contain at least $1.0 \times 10^9$ cfu per serving to be eligible to use a probiotic claim on the package (Health Canada 2009). The texture and thickness of yogurt products varies due to the non-fat solid content of the product (Heller 2001). Greek yogurt is characterized by a thick texture and a much higher protein content relative to other types of yogurt.

In North America, yogurt is the most common food source of probiotics with women almost twice as likely as men to consume probiotic yogurt (Granato, Branco et al. 2010).
f) **Dairy alternative products:**

While yogurt is one of the most popular probiotic food products (Granato, Branco et al. 2010), dairy alternatives are increasing in popularity for a variety of reasons including allergies, lifestyle choices, and the perceived health benefits offered (Makinen, Wanhalinna et al. 2014). While soy dominated the dairy alternative market early on, new products are becoming increasingly popular such as those derived from other plant sources like almond and coconut (Mintel 2011). These products are often fortified to mimic the vitamin and mineral content typically found in dairy products so as to offer a viable alternative to dairy products (Dharmasena and Capps Jr. 2014). Despite fortification, dairy alternatives still have marked nutritional differences from dairy products. In particular, the protein content and quality of most plant based products is significantly lower than that of dairy products (Makinen, Wanhalinna et al. 2014). Given the relative novelty of these commercial dairy alternative products, there is minimal research on their effects or benefits from a nutritional or health perspective.
6) Breakfast: Consumption Patterns, Composition and their role in Risks Associated with Metabolic Disorders

Regular breakfast consumption has been linked to lower weight and reduced weight gain over time in adults when investigated as part of large longitudinal health studies (Liu, Willett et al. 2003, Koh-Banerjee, Franz et al. 2004, Bazzano, Song et al. 2012). Numerous studies have also been conducted to look at the effect of breakfast on adolescents. Breakfast consumption in this population has been linked to improved academic performance (Rashidi, Mohammadpour-Ahranjani et al. 2007), lower BMI (Merten, Williams et al. 2009), a reduction in weight gain with age (Niemeier, Raynor et al. 2006) and has been shown to help predict regular breakfast consumption into young adulthood (Merten, Williams et al. 2009). Despite the known benefits of regular breakfast consumption, many individuals, both youth and adults, continue to skip breakfast (Huang, Hoerr et al. 1997, Reger, Nicklas et al. 1997, Rampersaud, Pereira et al. 2005, Song, Chun et al. 2005, Rashidi, Mohammadpour-Ahranjani et al. 2007). This is cause for concern from a nutritional perspective given that young adult breakfast skippers in the United States are less likely than breakfast consumers to meet nutritional RDA’s (Reger, Nicklas et al. 1997, Nicklas, Myers et al. 1998). With rising obesity rates and concerns about poor nutrition, breakfast offers an ideal meal to target in terms of improving population health and nutritional intake.

Breakfast also provides an opportunity to increase fibre intake and therefore total daily fibre consumption in individuals (Huang, Hoerr et al. 1997, Song, Chun et al. 2005) which is important in terms of population health given the observed benefits of fibre on weight
maintenance, particularly in older adults (Liu, Willett et al. 2003, Koh-Banerjee, Franz et al. 2004, Bazzano, Song et al. 2012), as well as cardiovascular disease risk (Brown, Rosner et al. 1999, Bazzano, He et al. 2003) and glucose tolerance (Wirstrom, Hilding et al. 2012). While there are a number of studies looking at middle aged men and women and children in relation to breakfast consumption (Liu, Willett et al. 2003, Koh-Banerjee, Franz et al. 2004, Barton, Eldridge et al. 2005, Rashidi, Mohammadpour-Ahranjani et al. 2007, Bazzano, Song et al. 2012), there is limited literature on breakfast consumption in young adults (Niemeier, Raynor et al. 2006, Merten, Williams et al. 2009). Additionally, as food consumption has been seen to differ by sex and age (Song, Chun et al. 2005), among other variables, a case can be made for the value in stratifying studies by gender to better understand the habits and preferences of each gender in terms of breakfast consumption.

a) **Fibre:**

The results of observational studies suggest that fibre intake is correlated with weight loss and a reduction in obesity risk, however experimental study results have not found conclusive evidence that fibre has a strong effect on intake or satiety (Smith and Tucker 2011). High fibre food consumption has been found to decrease hunger ratings significantly, however subsequent energy intake after a high fibre barley based test meal (12g fibre, 7.2g insoluble and 4.8g soluble) did not significantly differ from lower fibre whole wheat (5g fibre, 4.4g insoluble and .58g soluble) and rice based (1g fibre, 0.86g insoluble and 0.14g soluble) treatments (Schroeder, Gallaher et al. 2009). Fibre has been observed to delay gastric emptying in both liquid and solid meals and was also found to delay the postprandial return of hunger in one
study but, despite these results, this study found no difference in satiety or fullness between the high (20g fibre/100kcal) and low fibre (4g fibre/1000kcal) conditions (Benini, Castellani et al. 1995). When 6g of locust bean gum, a soluble fibre, was added to a test meal it resulted in significantly lowered rates of gastric emptying in healthy men and women (Darwiche, Bjorgell et al. 2003). Oatmeal was found to be associated with greater feelings of fullness, a lower desire to eat, and reduced prospective intake ratings in a four hour period after consumption as compared to a ready to eat breakfast cereal (Honey nut cheerios) in one study (Rebello, Johnson et al. 2013). It is thought that the high beta-glucan content of oatmeal may explain its effect on satiety (Rebello, Johnson et al. 2013). However, another study looking at the satiety provided by six different common breakfast items in India did not find a direct correlation between fibre content and satiety (Pai, Ghugre et al. 2005). Thus, further investigation into potential links between satiety and fibre is needed to obtain a more complete understanding of any relationship.

Despite the inconclusiveness of a satiety effect of fibre consumption, soluble fibre consumption has been well associated with a reduction in cardiovascular disease risk due to its cholesterol lowering effect (Brown, Rosner et al. 1999). In a crossover study significant reductions in total and LDL cholesterol levels were observed in healthy middle-aged individuals after a three week whole grain diet (23.1g fibre/day) as compared to a three week refined grain diet (9.8g fibre/day) (Giacco, Clemente et al. 2010). While the cholesterol lowering effect of soluble fibre in individuals is modest when clinically tested, the results are still significant enough to elicit approved government health claims for soluble fibre, specifically oat fibre, in cardiovascular disease risk reduction (Food Directorate Health Products and Food Branch 2010).
Fibre has also been linked to reduced diabetes risk due to its effect on glucose metabolism (Smith and Tucker 2011). A Finnish cohort study of 40-49 year old men and women without diabetes found a decreased incidence of type 2 diabetes at the 10 year follow-up in those with higher intakes of whole grains (Montonen, Knekt et al. 2003). An experimental study observed a 50% decrease in glucose response and a 30-40% decrease in insulin response in non-insulin dependent type two diabetics when increasing amounts (4-8.4g) of soluble fibre, in the form of beta glucan, was added to cereal with greater results seen in higher fibre treatment individuals (Tappy, Gugolz et al. 1996). 5g of sodium alginate, a soluble fibre, added to a meal was found to significantly reduce postprandial blood glucose and insulin levels in a study on men with well controlled non-insulin dependent type 2 diabetes, which was posited to be related to the slowed gastric emptying also seen with the sodium alginate treatment (Torsdottir, Alpsten et al. 1991). In longer term studies, insoluble fibre has been linked to improved insulin sensitivity in overweight and obese hyperinsulinemic individuals (Pereira, Jacobs Jr et al. 2002) as well as non-insulin dependent diabetic men (Pick, Hawrysh et al. 1996).

Disease and healthcare statistics have demonstrated that there is a need for continued improvements in the understanding of the relationship between food and its properties, satiety and blood glucose control. Current research correlates dairy proteins with measures of reduced intake through the actions of gastrointestinal hormones and improved blood glucose control. Research also shows potential impacts of other components and aspects of food, such as texture and fibre content, on satiety and food intake. Further research on the properties of
commercially available foods and the relationship to food intake, satiety, and blood glucose control can provide useful information for the management of diseases like obesity and type two diabetes.
Chapter 3: Rationale, Objectives, and Hypothesis

Rationale:

With economic and public health concerns related to increasing rates of overweight and obesity and limited success with current strategies to manage overweight and obesity, there is a continued need for research on alternative strategies. Foods with functional properties related to blood glucose control and satiety offer a potential avenue of further investigation.

There has been prior research demonstrating potential benefits of dairy products with regards to satiety and blood glucose control, however there is limited research comparing the effects of dairy products to non-dairy alternatives. Given the prevalence of breakfast skipping, there is merit in exploring commercially available foods that can be consumed for breakfast that also confer benefits in terms of satiety and blood glucose control. Existing research demonstrates beneficial effects of dairy products, including yogurt, on food intake and glycemic response, however existing studies explore the effects of dairy products consumed as a snack. As typical North American breakfast choices include dairy as a significant component of the meal, research on the effect of dairy as part of a meal is warranted. Furthermore, existing research has studied both men and women together. Given the known sex differences in intake and the hormonal influence on food intake in women, studying the effects of a treatment on satiety and food intake in men and women separately allows for close management of those potentially influencing factors.
Objectives:

Primary objectives:
The primary objective of this study was to investigate the effect of a breakfast of granola cereals served with dairy or non-dairy cultured products on (i) postprandial glycaemia, (ii) satiety and (iii) short-term food intake in young women.

Secondary objectives:
The secondary objective of this study was to assess short-term physical comfort levels along with postprandial insulin levels in the blood of young women over a two hour postprandial period after consuming a breakfast of granola cereal served with dairy or non-dairy cultured products.

Hypothesis:
Greek yogurt, in combination with granola cereal, consumed to satiation, will result in improved blood glucose control, and reduced short term food intake and subjective appetite, compared to the consumption of cultured coconut milk combined with granola cereal.
Chapter 4: Methods

Study Design:

This study used a within-subject balanced repeated-measures design to compare the effects of ad libitum consumption of Greek yogurt and cultured coconut milk combined with granola cereal on short-term food intake and postprandial glycaemia within two hours in young healthy women. At each experimental session, participants consumed one of three treatments: dairy or non-dairy matched for caloric density and available carbohydrate and a water control. Participants were randomly assigned the order of treatments to balance the experiment and consumed all treatments over the course of the study. A visual analogue scale for treatment palatability was completed. During each experimental session, seven blood samples were obtained to be analyzed for insulin and blood glucose response: a fasting sample and samples at 15, 30, 45, 60, 90 and 120 minutes after consumption of the treatment. Visual analogue scales measuring subjective appetite and physical comfort were completed every 15 minutes in the two hours between treatment consumption and the ad libitum pizza. Following the measurement of food intake with the pizza meal, visual analogue scales for treatment palatability, and post-meal appetite and physical comfort were completed.
Participants:

Participants were recruited through advertisements posted on Mount Saint Vincent University’s campus and Facebook page, and public resources such as the Halifax Kijiji website. Flyers advertising the study were distributed in public areas including grocery stores, libraries, and other university campuses. Participants were also recruited through the existing appetite lab participant database and word of mouth.

Thirty healthy women between the ages of 19 and 35 with a BMI of 18.5-24.9 kg/m² were recruited to participate. Exclusion criteria included having any diseases, irregular menses, smoking, taking medications known to influence sensory perception or gastrointestinal function, typically skipping breakfast, and having emotional, developmental or learning problems that would affect their ability to participate in the study as required. Individuals with known allergies to components of the treatments (dairy, coconut, wheat, soy, sesame) or who did not tolerate components of the treatments (dairy, coconut, blueberry) were also excluded as were those who were shown to have restrained eating patterns (as evaluated by the Eating Habits Questionnaire, Appendix D). The thirty participants recruited allowed for an anticipated attrition rate of 15% and was designed to enable the detection of an effect size of 150 kcal in pizza consumption using the sample size calculation with $\alpha=0.05$, $\beta=0.09$.

Individuals who expressed interest in partaking in the study were contacted by phone to provide further details about the study and to determine their eligibility for the study (appendix A). Women were then invited to Mount Saint Vincent University for further screening and to obtain written consent if they choose to participate in the study (appendix B and C). During the in-
person screening session, participants age, height, weight and body composition via bioelectrical impedance analysis using a Tanita Body Composition Analyzer (Tanita TBF-300A; Tanita Corporation of America, Inc, Arlington Heights, IL) were measured.

Participants were remunerated for each experimental session they attended and were also provided with compensation for transportation. The ethical components of this research study were reviewed by the University Research Ethics board and found to be in compliance with Mount Saint Vincent University’s Research Ethics Policy.

**Treatments:**

Treatments were consumed ad libitum by participants, one treatment per session, randomized in order, and consisted of:

1) water (energy-free control)

2) granola cereal (Kellogg’s Special K low fat granola, Kellogg Canada Inc.; ON, Canada) with plain Greek yogurt (Oikos 2%, Danone Inc; QC, Canada), blueberry flavouring (Light Double Fruit Blueberry Jam, Smuckers; ON, Canada), granulated sugar (Lantic Inc; QC, Canada), and water.

3) granola cereal (Kellogg’s Special K low fat granola, Kellogg Canada Inc.; ON, Canada) with plain Greek-style cultured coconut milk (So Delicious, Turtle Mountain; OR, United States) and blueberry flavouring (Light Double Fruit Blueberry Jam, Smuckers; ON, Canada), and water.
Treatments contained the same proportion of granola to cultured product and were developed to have the same amount of blueberry flavouring and similar energy density and grams of available carbohydrate.

**Treatment Composition:**

The caloric treatments contained the same proportion of granola to cultured product and were developed to have the same amount of blueberry flavouring and to have energy density and grams of available carbohydrate as close as possible (table 4.1),

Table 4.1: Experimental Treatment Nutritional Profile

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Dairy Treatment (per 100g)</th>
<th>Non-Dairy treatment (per 100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>149.9</td>
<td>145.9</td>
</tr>
<tr>
<td>Protein, g</td>
<td>9.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Fat, g</td>
<td>2.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>44.0</td>
<td>94.0</td>
</tr>
<tr>
<td>Total CHO, g</td>
<td>23.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Sugars, g</td>
<td>9.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Dietary fibre, g</td>
<td>2.0</td>
<td>5.6</td>
</tr>
<tr>
<td>AV CHO*</td>
<td>21.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Vitamin A, RE</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>96.8</td>
<td>162.8</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>2.8</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Available carbohydrate calculated as the difference between total carbohydrate and dietary fibre*
Participants arrived at the Mount Saint Vincent University Appetite Lab after fasting overnight for twelve hours except for water, which was permitted to be consumed up until one hour before arriving at the lab. Participants were requested to maintain the same or similar eating and exercise habits the day before each study session and to arrive at the lab at the same time for each session to minimize variability among their results. To evaluate compliance to these requests, participants completed a food intake and activity questionnaire at the beginning of
each session. Additionally, to minimize the effect of hormonal fluctuations on study outcomes, participants were scheduled to attend each session during the follicular phase in their menstrual cycle which permitted a maximum of two sessions per month with a one week washout period between experimental sessions.

Upon arriving at the lab, participants completed a baseline questionnaire (Recent Food Intake and Activity) and visual analogue scales (Motivation to Eat, Energy and Fatigue, and Physical Comfort). An IV catheter was inserted into the antecubital vein by a nurse and a fasting venous blood sample was obtained. The IV catheter remained in the participants arm for the duration of the experimental session. A droplet of blood from the fasting blood sample was placed on a blood glucose monitoring strip (Accu-Chek Compact test strip, Roche Diagnostics; Mannheim, Germany) inserted into a blood glucose monitoring system (Accu-Chek Compact Plus Blood Glucose Monitoring System, Roche Diagnostics; Mannheim, Germany) to evaluate compliance to the overnight fast. Individuals whose blood glucose concentration was >6mmol/L were unable to participate in an experimental session that day and were requested to reschedule.

Participants were then provided with the treatment to consume ad libitum until satiation. The two caloric treatments were eaten and served with water and water was served alone for the control treatment. Participants had 15 minutes to consume the treatment. Treatments were provided to the participants in a balanced order with one treatment being provided at each experimental session. A randomized balanced treatment order was used to eliminate the potential for confounding of results due to a treatment order or session effect. The palatability of the treatment was evaluated using a visual analogue scale. The quantity of treatment
consumed by each individual was determined by the weight of treatment consumed and nutritional information was calculated based on the manufacturers information. The quantity of water consumed along with the caloric treatments was also measured.

For two hours following consumption, visual analogue scales (Motivation to Eat, Energy and Fatigue, and Physical Comfort) were completed every fifteen minutes. Blood samples were obtained during this two hour period at 15, 30, 45, 60, 90, and 120 minutes after consumption of the treatment.

Participants were served an ad libitum pizza meal two hours after the treatment was consumed and were asked to eat pizza until “comfortably full”. A glass of water, weighed prior to and after the pizza meal was consumed, were provided to each participant with the pizza. Once the participant finished their pizza meal, visual analogue scales were completed for Pizza Palatability, Motivation to Eat, Energy and Fatigue, and Physical Comfort and the participant was free to leave the lab. The quantity of the pizza meal consumed by each participant was determined based on the weight consumed and detailed nutritional information for the pizza meal was calculated based on the manufacturers information.

**Blood Collection:**

Prior to the start of the experimental session, participant blood glucose was checked to determine their baseline plasma glucose concentration and to confirm compliance to the overnight fast. The participants fasting blood glucose was sampled by the nurse and a drop of blood from that sample was used to test their blood glucose levels on site. If the participants
blood glucose measured >6 mmol/L they were ineligible to participate in the study that day and were rescheduled for another day.

All blood sampling was carried out using aseptic techniques by a nurse licensed in the Province of Nova Scotia. To minimize risk of blood contact, the nurses wore non-latex gloves to perform the blood draws. The nurse inserted a catheter into the antecubital vein which remained in the participants arm for the duration of the experimental session.

Blood samples of 5.0mL each were collected in serum separating tubes and of 2.0mL each were collected in sodium heparin tubes connected to the catheter and both samples were obtained at 15, 30, 45, 60, 90, and 120 minutes after consumption of the treatment. Serum separating tubes used were 5.0mL BD Vacutainer® SST tubes (Becton, Dickinson and Company, ON, Canada). Sodium heparin tubes used were 2.0mL BD Vacutainer® sodium heparin 33 USP tubes (Becton, Dickinson and Company, ON, Canada).

The blood collection system that was used was a shielded intravenous catheter (BD Insyte Autoguard™ Winged) connected to both a Luer-Access split septum device (BD Q-Syte™) and a Luer-Lok™ access device (BD Vacutainer®).

The serum separating tubes and sodium heparin tubes were labeled with the participant ID, treatment ID, date, and time point. After blood was drawn, serum separating tubes were held at room temperature for 30 minutes after gentle hand inversion five times. After 30 minutes of standing to allow for clotting, the tubes were centrifuged at 1300g (RCF) for 10 minutes at 15°C and aliquoted into 0.5mL labeled microtubes. The samples were divided to allow for analyzing for both glucose (3 microtubes of 0.5mL) and insulin (3 microtubes of 0.5mL). Microtubes were
stored in a -80°C freezer until analyzed. After blood was drawn, sodium heparin tubes were covered in a foil wrapper to prevent light contamination and gently inverted by hand eight to ten times. The tubes were held on ice and centrifuged as soon as possible or immediately centrifuged at 1300g (RCF) for 10 minutes at 4°C and aliquoted into five 0.5mL amber microtubes. Two of the five microtubes were prefilled with 4.5uL of 50% formic acid. Amber microtubes were stored in a -80°C freezer to be used for secondary analysis.

**Blood Analysis:**

Blood samples were analyzed for blood glucose and insulin over the 120 minute sampling period for all three treatments. Blood glucose samples were analyzed with the YSI 2300 Stat Plus Glucose Analyzer (YSI Incorporated, Yellow Springs, Ohio). Insulin analysis was conducted using the Insulin ELISA immunoassay (80-INSHU-E01.1, Alpco Diagnostics, New Hampshire, United States).

The data from the analysis was used to determine the effect of each treatment on postprandial blood glucose and insulin response.

**Food Intake:**

Overall participant food intake was measured by weighing the amount of the treatment, pizza, and water consumed during the experimental session. These quantities were obtained by weighing the dishes after the participant finished eating and subtracting that weight from the
weight of the dishes plus food prior to being served to the participant. Weights were used to calculate specific nutrient intake (calories, available carbohydrates etc.) based on the manufacturers nutrition label for each product.

**Pizza Meal:**
The pizza meal served two hours after treatment consumption was used as the *ad libitum* measure of subsequent energy intake so as to be able to measure the satiety effect of the treatments. In the screening questionnaires, participants indicated their preference of three varieties of pizza (Cheese, Deluxe, and Pepperoni). Their preferred type of pizza was then provided to them as their pizza meal (McCain Foods Ltd, NB, Canada) for all three experimental sessions. Pizza was chosen to measure food intake due to its popularity and high level of palatability to minimize the possibility of participant dislike of the food affecting the quantity consumed. Participants were asked to eat the pizza until “comfortably full” and were given a period of 20 minutes to consume the meal. The pizzas were prepared according to the manufacturer’s instructions and were weighed prior to and after the participant had finished eating. Water was also provided to participants for consumption with the pizza meal.

**Subjective Measures:**
Visual Analogue Scales (VAS) were used to measure a variety of relevant subjective factors related to appetite research including treatment palatability, motivation to eat, and physical comfort. The scales posed a question (ie. “How did you like the texture of the treatment?”),
with a 100mm line below it with opposing answers at each end (ie. “Not at all” vs. “Very much”). Participants were asked to mark an x across the line at the point that most accurately reflected their current feelings. A measurement was taken from the left end of the line to the marked x which was the participants score for that particular question. Several VAS were presented to the participant throughout the experimental session. A palatability VAS was provided following the consumption of the treatment (appendix H) as well as the pizza meal (appendix L). Motivation to eat, energy and fatigue, and physical comfort VAS (appendix I-K) were completed at the participants arrival to the lab, every 15 minutes for the two hours following consumption of the treatment, and following consumption of the pizza meal.

VAS are used extensively in appetite research and have been determined to be valid measures of appetite, desire to eat, hunger, meal appetite, and anticipated quantity of food consumed and correlate to later food intake, particularly in laboratory settings using within-subject, repeated-measures experimental designs in addition to physiological measures (Flint, Raben et al. 2000, Stubbs, Hughes et al. 2000), as this study employed.

**Statistical Analysis:**

Statistical analysis was conducted using SAS version 9.3 (Statistical Analysis Systems, SAS Institute, Cary, NC, USA). Blood glucose and insulin concentrations were calculated for the two hour experimental period and total area under the curve (tAUC) values were determined using the participants fasting blood glucose value as the baseline level.
ANOVA tests to compare treatments, time, blood insulin, and blood glucose values were conducted. A p-value of less than 0.05 was considered statistically significant. When a significant treatment effect was observed, the differences between the treatments were further analyzed using Tukey’s HSD post hoc analysis.
Chapter 5: Results

Participant Attributes:

Twenty four women between 19 and 28 years old completed the study. The participants had an average BMI of 22.13. Baseline participant attributes are outlined in table 5.1.

<table>
<thead>
<tr>
<th>Participant Attribute</th>
<th>Mean with standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.71 ± 2.53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.00 ± 6.20</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.64 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.13 ± 1.49</td>
</tr>
<tr>
<td>Fat Mass</td>
<td>14.36 ± 5.99</td>
</tr>
<tr>
<td>Fat Free Mass</td>
<td>45.83 ± 4.26</td>
</tr>
<tr>
<td>Eating Habits Score</td>
<td>1.05 ± 0.43</td>
</tr>
</tbody>
</table>
Blood Glucose

There was a significant effect of time (P<.0001), treatment (P<.0001), and a treatment by time interaction (P<.0001) on mean two-hour blood glucose concentrations (table 5.3). Analysis of treatment by time interactions revealed a significant difference between dairy and non-dairy treatments at 30 minutes (P=.0059), 45 minutes (P<.0001) and 60 minutes (P<.0001) with the blood glucose levels lower in the dairy treatment compared to the non-dairy treatment at all time points (figure 5.1).

There was a significantly lower overall blood glucose response to the dairy compared to the non-dairy treatments (P=.0001), and non-dairy and water treatments (P<.0001) using the Tukey-Kramer adjustment. The mean 2 hour dairy treatment was observed to be 9.3% lower than the non-dairy treatment. No session effect of blood glucose was seen for any of the treatments.

The analysis of treatments using a total area under the curve (tAUC) revealed that the dairy treatment had a 10.4% lower tAUC compared to the non-dairy treatment (table 5.3).

Table 5.3. Blood Glucose Response

<table>
<thead>
<tr>
<th></th>
<th>Dairy Treatment</th>
<th>Non-Dairy Treatment</th>
<th>Water Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 2 hour Blood Glucose (mmol/L)</td>
<td>4.6 ± 0.08a</td>
<td>5.07 ± 0.09b</td>
<td>4.40 ± 0.03a</td>
</tr>
<tr>
<td>Mean 2 hour Blood Glucose: Change from Baseline (mmol/L)</td>
<td>-0.03 ± 0.67</td>
<td>-0.63 ± 0.63</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>Total Area Under the Curve (mmol/L)</td>
<td>539.48 ± 26.86</td>
<td>601.95 ± 29.87</td>
<td>526.2 ± 33.27</td>
</tr>
</tbody>
</table>

*Data presented as mean ± standard deviation, n=24*
**One-way ANOVA with Tukey Kramer post-hoc test.**

*Different superscripts represent significant differences between treatments (P<0.05)*

Figure 5.1 Blood Glucose Concentration Over 120 Minutes

![Blood Glucose Graph](image)

*Data presented as mean ± standard deviation, n=24; different superscripts represent significant differences between treatments (P<0.05)*

**Blood Insulin**

An effect of time (P<.0001), treatment (P<.0001), and a treatment by time interaction (P<.0001) was observed when mean blood insulin levels were analyzed (table 5.4). Treatment by time interaction analysis showed a significant difference between dairy and non-dairy treatments at 15 minutes (P=.0123), 30 minutes (P<.001), and 45 minutes (P=.0287) with non-dairy treatment values being lower at all time points compared to the dairy treatment insulin levels (figure 5.2).
Analysis showed a significant difference between overall blood insulin levels between the dairy and non-dairy treatments (P<.0001) with the dairy treatment having a 58.1% greater mean insulin response compared to the non-dairy treatment. A significant difference was also seen between the dairy and water treatments (P<.0001), and non-dairy and water treatments (P<.0001). No session effect was observed with blood insulin levels for any of the treatments.

No effect of treatment, session, or treatment by session interaction on insulin response was observed at baseline. An effect of treatment (P<.0001) on insulin response at 15 minutes was observed between the dairy and non-dairy treatments (P=.0002), dairy and water treatments (P<.0001), and non-dairy and water treatments (P=.0004). A treatment effect on insulin response at 30 minutes (P<.0001) was observed between the dairy and non-dairy treatments (P=.0010), dairy and water treatments (P<.0001) and non-dairy and water treatments (P<.0001). An effect of treatment on insulin response was observed at 45 minutes (P<.0001) between the dairy and non-dairy treatments (P=.0421), dairy and water treatments (P<.0001), non-dairy and water treatments (P=.0001). A treatment effect at 60 minutes on insulin response was seen (P<.0001) with significant differences observed between the two caloric treatments and water (P<.0001 for both). An effect of treatment on insulin response at 90 minutes (P<.0001) was observed between the dairy and non-dairy treatments (P=.00013), dairy and water treatments (P<.0001), and the non-dairy and water treatments (P=.0079). A treatment effect on insulin response at 120 minutes (P<.0001) was observed with significant differences seen between the dairy and non-dairy treatments (P=.0189), dairy and water treatments (P<.0001), and non-dairy and water treatments (P=.0416).
A comparison of treatments using total area under the curve (tAUC) showed a 59% higher insulin response for the dairy treatment compared to the non-dairy treatment (table 5.4).

Table 5.4. Blood Insulin Response

<table>
<thead>
<tr>
<th></th>
<th>Dairy Treatment</th>
<th>Non-Dairy Treatment</th>
<th>Water Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 2 hour Blood Insulin (µlU/mL)</td>
<td>41.33 ± 2.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.14 ± 2.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.98 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean Blood Insulin Change from Baseline (µlU/mL)</td>
<td>-39.45 ± 21.70</td>
<td>-21.70 ± 12.88</td>
<td>1.28 ± 0.64</td>
</tr>
<tr>
<td>Total Area Under the Curve (µlU /mL)</td>
<td>5103.83 ± 262.18</td>
<td>3208.13 ± 174.0</td>
<td>699.23 ± 38.92</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation, n=24

One-way ANOVA with Tukey Kramer post-hoc test.

Different superscripts represent significant differences between treatments (P<0.05)
Figure 5.2 Blood Insulin Response Over 120 Minutes

*Data presented as mean ± standard deviation, n=24; different superscripts represent significant differences between treatments (P<0.05)

Ad Libitum Food Intake

Analysis of the nutritional content of the caloric treatments revealed significant differences between the dairy and non-dairy treatments in amounts of protein (P<.001), total carbohydrate (P=.008), and fibre (P<.001). The dairy treatment provided significantly more protein compared to the non-dairy treatment and the non-dairy treatment provided significantly higher amounts of total carbohydrate and fibre compared to the dairy treatment.

Analysis of pizza intake revealed an effect of treatment on pizza intake (P=.003). No significant difference in pizza intake between the dairy and non-dairy treatments was observed, however
there was a significant difference in pizza intake between the dairy and water treatments (P=.0078) and the non-dairy and water treatments (P=.0075). No effect of session on pizza intake was seen (table 5.5).

There was an effect of treatment (P<0.001), session (P=.0429), and session by treatment (P=.0319) on cumulative food intake. The dairy treatment showed a 6.7% higher caloric intake compared to the non-dairy treatment, however this was not a significant difference (P=.59).

There was, however, a significant difference between the dairy and water treatments (P<.0001) and the non-dairy and water treatments (P<.0001) (table 5.5 and figure 5.5).

Table 5.5 Mean Intake

<table>
<thead>
<tr>
<th></th>
<th>Dairy Treatment</th>
<th>Non-Dairy Treatment</th>
<th>Water Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Food Intake (kcal)</td>
<td>332 ± 85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>311 ± 84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>19.91 ± 5.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.22 ± 1.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>6.64 ± 1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.22 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>53.09 ± 13.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.10 ± 16.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>19.91 ± 5.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.66 ± 5.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>3.32 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.44 ± 3.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Available Carbohydrate (g)</td>
<td>46.45 ± 11.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.66 ± 12.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td>Pizza Intake (kcal)</td>
<td>365 ± 133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>360 ± 151&lt;sup&gt;a&lt;/sup&gt;</td>
<td>448 ± 221&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cumulative Food Intake (treatment + pizza meal, kcal)</td>
<td>697 ± 168&lt;sup&gt;a&lt;/sup&gt;</td>
<td>671 ± 174&lt;sup&gt;a&lt;/sup&gt;</td>
<td>448 ± 221&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water Intake (g)</td>
<td>441 ± 199&lt;sup&gt;a&lt;/sup&gt;</td>
<td>444 ± 133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>530 ± 191&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation, n=24

Two-way ANOVA with Tukey Kramer post-hoc test.

Different superscripts represent significant differences between treatments (P<0.05)
Figure 5.3 Mean Treatment Intake (kcal)

*Data presented as mean ± standard deviation, n=24; different superscripts represent significant differences between treatments (P<0.05)

Figure 5.4 Mean Pizza Intake (kcal)

*Data presented as mean ± standard deviation, n=24; different superscripts represent significant differences between treatments (P<0.05)
Figure 5.5 Mean Cumulative Food Intake (kcal)

*Data presented as mean ± standard deviation, n=24; different superscripts represent significant differences between treatments (P<0.05)

Figure 5.6 Mean Cumulative Water Intake (g)

*Data presented as mean ± standard deviation, n=24, different superscripts represent significant differences between treatments (P<0.05)
Palatability

Analysis revealed no significant effect of pleasantness on treatment, session, or treatment by session interaction (table 5.6 and figure 5.7). The dairy treatment mean pleasantness scores were 4.1% higher than the non-dairy treatment scores, however this difference was not significant (table 5.6 and figure 5.7). There was an effect of treatment on taste (P<.0001) but no effect of session or treatment by session interaction (table 5.6). A 6.1% higher mean taste score was observed for the dairy treatment compared to the non-dairy treatment, however this difference was not significant (P=.77). A significant difference in taste scores was observed between the dairy and water treatments (P<.0001) and the non-dairy and water treatments (P<.0001) (figure 5.8). Analysis showed a significant effect of treatment on texture (P=.0144) but no significant effect of session or treatment by session interaction (table 5.6 and figure 5.9). The dairy treatment texture scores were 4.6% higher than the non-dairy treatment scores, however this difference was not significant (P=.71). No significant difference was observed in texture scores between the non-dairy and water treatments (P.08), however a significant difference was seen between the dairy and water treatments (P=.0135) (table 5.6 and figure 5.9). Analysis revealed no significant effect between pizza pleasantness scores and treatment, session, or treatment by session interaction (table 5.6 and figure 5.10).
Table 5.6 Palatability Scores

<table>
<thead>
<tr>
<th></th>
<th>Dairy Treatment</th>
<th>Non-Dairy Treatment</th>
<th>Water Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pleasantness (mm)</td>
<td>80.19 ± 2.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.92 ± 3.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.75 ± 5.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean taste (mm)</td>
<td>82.75 ± 2.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.73 ± 3.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.17 ± 5.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean texture (mm)</td>
<td>83.31 ± 2.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.50 ± 2.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>67.85 ± 5.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean pizza pleasantness (mm)</td>
<td>82.71 ± 2.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.54 ± 2.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.56 ± 4.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation, n=24

One-way ANOVA with Tukey Kramer post-hoc test.

Different superscripts represent significant differences between treatments (P<0.05)

Figure 5.7 Mean Treatment Pleasantness Rating

*Data presented as mean ± standard deviation, n=24; Ratings based on VAS score on 100mm line*
Figure 5.8 Mean Treatment Taste Rating

*Data presented as mean ± standard deviation, n=24; Ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)

Figure 5.9 Mean Treatment Texture Rating

*Data presented as mean ± standard deviation, n=24; Ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)
Measures of Subjective Appetite

Average Appetite:

Average appetite scores were calculated using change from baseline scores of desire to eat, hunger, fullness, and potential food consumption. Analysis showed a significant effect of treatment ($P<.0001$), time ($P<.0001$), and treatment by time interaction ($P<.0001$). No effect of session ($P=.08$) was observed. The mean scores for the non-dairy treatment were 9.4% higher compared to the dairy treatment, however this was not a significant difference ($P=.56$). The dairy and water treatments ($P<.0001$) and non-dairy and water treatments ($P<.0001$) were observed to be significantly different. Average appetite scores were lower at all time points after baseline for the non-dairy treatment compared to the water and dairy treatments but they were not significantly different from the dairy treatment scores at any time point (figure...
5.11). The dairy and water treatment and non-dairy and water treatment average appetite scores differed significantly at all time points after baseline (figure 5.11).

Comparison of total area under the curve showed the non-dairy treatment had an 11.3% greater area under the curve compared to the dairy treatment (table 5.8).

**Desire to Eat:**

Desire to eat scores were calculated as change from baseline. Analysis of desire to eat VAS scores showed an effect of treatment (P<.0001), time (P<.0001) and session (P=.0354) as well as treatment by time (P<.0001), and session by time (P=.0023), and session by treatment (P=.0026) interactions. Mean non-dairy treatment scores were 15.3% higher than dairy treatment scores, however this was not a significant difference (P=.43). Significant differences were seen between the dairy and water treatment scores (P<.0001) and the non-dairy and water treatment scores (P<.0001). The non-dairy treatment showed lower desire to eat scores at each time point, however no time point had significant differences in scores between the dairy and non-dairy treatments. Significant differences between the dairy and water treatments were seen at time points 15, 30, 45, 60, 75, and 90 (figure 5.12). Significant differences between the non-dairy and water treatments were seen at time points 15, 30, 45, 60, 75, 90, 105, and 120 (figure 5.12).

Analysis comparing the total area under the curve showed the dairy treatment had a 15.5% lower total area under the curve compared to the non-dairy treatment (table 5.8).
Hunger:

VAS scores for hunger were calculated as change from baseline. Analysis of VAS hunger scores found a significant effect of time (P<.0001) and treatment (P<.0001). Treatment by time (P<.0001) and session by treatment interactions (P=.0072) of significance were also observed. No effect of session was seen (P=.34). The non-dairy treatment showed a 13.2% greater mean score compared to the dairy treatment, however this was not significant (P=.39). Significant differences were seen between the dairy and water treatments (P<.0001) and the non-dairy and water treatments (P<.0001). The non-dairy treatment resulted in consistently lower VAS hunger scores compared to the dairy and water treatments, however there was no significant difference between the dairy and non-dairy treatment scores at any time point (figure 5.13). Significant differences were seen between the dairy and water treatments at time points 15, 30, 45, 60, 75, 90, and 105 and between the non-dairy and water treatments at time points 15, 30, 45, 60, 75, 90, 105, and 120 (figure 5.13).

When total area under the curve for all treatments were compared, the area was 13.6% greater for the non-dairy treatment compared to the dairy treatment (table 5.8).

Fullness:

Fullness VAS scores were calculated as change from baseline. When analyzed, a significant effect of time (P<.0001), treatment (P<.0001), and session (P=.0283) were observed on VAS fullness scores. Significant effects of treatment by time (P<.0001) and session by treatment (P=.0018) were also seen. The mean dairy treatment scores were 4.1% higher than the mean non-dairy treatment scores, however this was not a significant difference (P=.89). The dairy and water treatments (P<.0001) and non-dairy and water treatments (P<.0001) were seen to
have significantly different scores. Similarly, no time points were seen to be significantly different between the dairy and non-dairy treatments (figure 5.14). Significant differences were observed between the dairy and water treatments and the non-dairy and water treatments at all time points after baseline (figure 5.14).

When total area under the curve was compared, the dairy treatment had a 4.6% greater area compared to the non-dairy treatment (table 5.8).

**Potential Food Consumption:**

VAS scores for potential food consumption were calculated as change from baseline. When analyzed, significant effects of time (P<.0001), treatment (P<.0001), and treatment by time interactions (P<.0001) were observed. A borderline session effect was seen (P=.056). A 9.5% greater mean potential food consumption score was observed for the non-dairy treatment compared to the dairy treatment, however this was not significant (P=.82). The scores for the dairy and water treatments (P<.0001) and the non-dairy and water treatments (P<.0001) were significantly different. Dairy and non-dairy treatment potential food consumption scores were not significantly different at any time point while both dairy and water treatment and non-dairy and water treatment scores were found to be significantly different at all time points after baseline (figure 5.15).

When potential food consumption total area under the curve was compared, the non-dairy treatment had an 10.4% larger area compared to the dairy treatment (table 5.8).
Table 5.7 Subjective Appetite Measures

<table>
<thead>
<tr>
<th></th>
<th>Dairy</th>
<th>Non Dairy</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Appetite (mm)</td>
<td>37.33 ± 37.16</td>
<td>41.22 ± 37.22</td>
<td>73.19 ± 32.86</td>
</tr>
<tr>
<td>Average Appetite Change from Baseline (mm)</td>
<td>-4.61 ± 12.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.37 ± 12.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.55 ± 5.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Desire to Eat (mm)</td>
<td>33.59 ± 25.19</td>
<td>39.65 ± 26.16</td>
<td>73.01 ± 15.02</td>
</tr>
<tr>
<td>Desire to Eat Change from Baseline (mm)</td>
<td>-20.67 ± 43.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-26.11 ± 43.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.59 ± 26.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>32.81 ± 24.40</td>
<td>37.78 ± 25.77</td>
<td>69.68 ± 16.79</td>
</tr>
<tr>
<td>Hunger Change from Baseline (mm)</td>
<td>-16.63 ± 40.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-22.73 ± 41.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.06 ± 23.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>58.28 ± 22.84</td>
<td>55.91 ± 25.38</td>
<td>19.22 ± 14.44</td>
</tr>
<tr>
<td>Fullness Change from Baseline (mm)</td>
<td>35.79 ± 27.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.57 ± 27.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73 ± 18.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potential Food Consumption (mm)</td>
<td>39.21 ± 21.87</td>
<td>43.34 ± 22.33</td>
<td>69.26 ± 14.09</td>
</tr>
<tr>
<td>Potential Food Consumption Change from Baseline (mm)</td>
<td>-15.24 ± 35.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-15.99 ± 37.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.86 ± 22.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation, n=20

Two-way ANOVA with Tukey Kramer post-hoc test.

Different superscripts represent significant differences between treatments (P<0.05)
Table 5.8 Subjective Appetite Measures tAUC

<table>
<thead>
<tr>
<th></th>
<th>Dairy</th>
<th>Non Dairy</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Appetite tAUC</td>
<td>4097.0 ± 156.65</td>
<td>4620.83 ± 155.72</td>
<td>8763.23 ± 82.60</td>
</tr>
<tr>
<td>Desire to Eat tAUC</td>
<td>3697.69 ± 160.91</td>
<td>4377.94 ± 181.36</td>
<td>8731.69 ± 89.24</td>
</tr>
<tr>
<td>Hunger tAUC</td>
<td>3621.19 ± 178.59</td>
<td>4191.94 ± 175.83</td>
<td>8377.39 ± 97.53</td>
</tr>
<tr>
<td>Fullness tAUC</td>
<td>7359.19 ± 153.42</td>
<td>7030.69 ± 145.87</td>
<td>2352.56 ± 82.87</td>
</tr>
<tr>
<td>Potential Food Consumption tAUC</td>
<td>4430.25 ± 139.72</td>
<td>4944.94 ± 123.71</td>
<td>8295.79 ± 63.03</td>
</tr>
</tbody>
</table>

Subjective Appetite Measures

Figure 5.11 Average Appetite:

N=20; Data presented as mean ± standard deviation; different superscripts represent significant differences between treatments (P<0.05)
Figure 5.12 Desire to Eat:

*N=20; Data presented as mean ± standard deviation; ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)*
Figure 5.13 Hunger:

N=20; Data presented as mean ± standard deviation; ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)
Figure 5.14 Fullness:

N=20; Data presented as mean ± standard deviation; ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)
Figure 5.15 Potential Food Consumption:

N=20; Data presented as mean ± standard deviation; ratings based on VAS score on 100mm line; different superscripts represent significant differences between treatments (P<0.05)
Correlation:

**Food Intake and Blood Glucose:**

No significant correlation was seen between baseline blood glucose levels and treatment intake (P=0.29), pizza intake (P=0.67), or cumulative food intake (P=0.25) (table 5.9). Likewise, no significant correlation was observed between blood glucose values at 120 minutes and treatment intake (P=0.69), pizza intake (P=0.64), and cumulative food intake (P=0.94) (table 5.9).

**Table 5.9: Correlation of Food Intake and Blood Glucose**

<table>
<thead>
<tr>
<th></th>
<th>Treatment Intake</th>
<th>Pizza Intake</th>
<th>Cumulative Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BG</td>
<td>r=0.13</td>
<td>r=0.05</td>
<td>r=0.14</td>
</tr>
<tr>
<td>BG 120 min</td>
<td>r=-0.05</td>
<td>r=0.06</td>
<td>r=0.01</td>
</tr>
</tbody>
</table>

N=24; *indicates a statistically significant correlation (P<.05)

**Food Intake and Blood Insulin:**

Correlation analysis found no significant correlation between baseline insulin levels and treatment intake (P=0.75), pizza intake (P=0.61), and cumulative food intake (P=0.87) (table 5.10). A significant (P<.0001) moderately positive correlation was observed between insulin levels at 120 minutes and treatment intake. The correlation between insulin levels and cumulative food intake was also positive and significant (P.011) though low in strength (table 5.10). There was no significant correlation between insulin at 120 minutes and pizza intake (P=0.55).
Table 5.10: Correlation of Food Intake and Blood Insulin

<table>
<thead>
<tr>
<th></th>
<th>Treatment Intake</th>
<th>Pizza Intake</th>
<th>Cumulative Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Insulin</td>
<td>r=0.04</td>
<td>r=-0.06</td>
<td>r=-0.02</td>
</tr>
<tr>
<td>Insulin 120 min</td>
<td>r=0.46*</td>
<td>r=-0.07</td>
<td>r=0.30*</td>
</tr>
</tbody>
</table>

N=24; *indicates a statistically significant correlation (P<.05)

**Food Intake and Measures of Appetite:**

A significant strong negative correlation was observed between the average desire to eat scores and treatment intake (P<.0001), a significant moderate positive correlation was observed between the average desire to eat score and pizza intake (P=.0001), and the correlation between average desire to eat scores and cumulative food intake was not significant (P=0.11) (table 5.11).

The correlation between mean hunger scores and treatment intake was significant, negative and strong (P<.0001) whereas the correlation with pizza intake was significant, positive and moderate (P=.0001). A significant correlation was not observed between mean hunger scores and cumulative food intake (P=0.24) (table 5.11).

Mean fullness scores significantly correlated with all food intake measures. The correlation was strongly positive with treatment intake (P<.0001), low and negative with pizza intake (P=.0001), and low and positive with cumulative food intake (P=0.015).
Potential food consumption scores had a strong, negative, significant (P<.0001) correlation with treatment intake, moderately positive significant (P<.0001) correlation with pizza intake, and the correlation was not significant with cumulative food intake (P=0.35) (table 5.11).

Average appetite scores had a significant (P<.0001), strong, negative correlation with treatment intake and a significant (P=.0002) moderately positive correlation with pizza intake. The correlation between average appetite and cumulative food intake was not significant (P=0.11) (table 5.11).

Table 5.11: Correlation of Food Intake and Measures of Appetite

<table>
<thead>
<tr>
<th></th>
<th>Treatment Intake</th>
<th>Pizza Intake</th>
<th>Cumulative Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Desire to Eat</td>
<td>r=-0.77*</td>
<td>r=0.45*</td>
<td>r=-0.21</td>
</tr>
<tr>
<td>Mean Hunger</td>
<td>r=-0.73*</td>
<td>r=0.48*</td>
<td>r=-0.15</td>
</tr>
<tr>
<td>Mean Fullness</td>
<td>r=0.81*</td>
<td>r=-0.36*</td>
<td>r=0.31*</td>
</tr>
<tr>
<td>Mean Potential Food Consumption</td>
<td>r=-0.72*</td>
<td>r=0.46*</td>
<td>r=-0.12</td>
</tr>
<tr>
<td>Mean Average Appetite</td>
<td>r=-0.78*</td>
<td>r=0.46*</td>
<td>r=-0.21</td>
</tr>
</tbody>
</table>

N=20; *indicates a statistically significant correlation (P<.05)
Chapter 6: Discussion

The results of this study support the hypothesis: that the dairy treatment would result in improved blood glucose control compared to the non-dairy treatment. The overall blood glucose response to the dairy treatment was significantly lower than that of the non-dairy treatment which also resulted in a lower total area under the curve for the dairy treatment. Specific time points that were significantly lower for the dairy treatment were 30 minutes, 45 minutes, and 60 minutes. This reduced blood glucose response to the dairy treatment fits with what has been seen in other experimental studies looking at postprandial blood glucose concentrations where the response was posited to be related to the presence of milk proteins (Panahi, Al Khoury et al. 2013, Panahi, Luhovyy et al. 2013). Greek yogurt containing 9.2g protein/100g treatment (table 4.1) was used in this study for the dairy treatment, providing a significant contribution of dairy protein to potentially influence blood glucose response. As would be expected due to the lack of calorie consumption from the treatment, the water treatment resulted in very little glucose response.

Similarly, the mean insulin response between all treatments was significant. However, the dairy treatment had a significantly higher insulin response compared to the non-dairy treatment and the water treatment, which had the lowest insulin response. Time points with significantly higher insulin levels between the dairy and non-dairy treatments were 15, 30 and 45 minutes. These results are consistent with literature that suggests that dairy consumption, particularly the consumption of whey proteins, leads to increased insulin levels (Luhovyy, Akhavan, et al. 2007).
The study results did not, however, support the hypothesis that the dairy treatment would result in a lower food intake compared to the non-dairy treatment. In fact, the mean cumulative food intake (treatment plus pizza meal) for the dairy treatment sessions was slightly, though nonsignificantly, higher than that of the non-dairy treatment sessions. Given that the treatment intake and pizza meal were separated by only two hours, participants may not have had sufficient time to become ready for a subsequent meal, resulting in a reduction in intake at the pizza meal. This is supported by the VAS measures of appetite. The measures of desire to eat, hunger, fullness, and potential food consumption did not reach the baseline levels at 120 minutes for either the dairy and non-dairy treatments suggesting that participant perceptions of hunger were lower at that time than prior to consuming the treatment.

Assuming an intake of 1500kcal/day for the young adult female participants would put an average meal at approximately 500 kcal, however, the average pizza intake for both the dairy and non-dairy sessions was close to 360 kcal, suggesting that participants were either restricting their intake or were not hungry enough to consume an amount that would constitute a regular meal. It would be reasonable, therefore, to assume that the treatment meals would be more in line with the 500kcal estimate given that the participants had fasted overnight prior to the meal. The average intake, however, was 331.81kcal of the dairy treatment and 311.07kcal of the non-dairy treatment (table 5.5). Given that breakfast tends to be a smaller meal compared to lunch and supper meals, or skipped entirely, in North America, these numbers may not be that abnormal, however that observation makes the low intake at the pizza meal even more significant. Given the age and gender of the participants and the fact that the meal consisted of pizza which is commonly considered an ‘unhealthy’ food, perhaps
participants intentionally or unintentionally restricted their intake at the pizza meal based on perceptions of what and how much they should be eating.

An analysis of the nutrients present in the caloric treatments revealed significant differences between the dairy and non-dairy treatments in the amounts of protein, total carbohydrates and fibre consumed. As was expected based on the nutritional profile of the treatments, dairy treatment consumption resulted in significantly higher protein (19.91g) compared to the non-dairy treatment (6.22g), while the non-dairy treatment provided significantly more fibre (12.44g) than the dairy treatment (3.32g) which resulted in the non-dairy treatment also being significantly higher for total carbohydrate (59.10g) compared to the dairy treatment (53.09g).

The quantity of protein consumed by the participants in this study is less than that seen in the high protein treatments in research evaluating the effect of high and low protein meals, where the high protein treatments contained 73g protein/463g treatment and 39g protein/265g treatment (Vandewater and Vickers 1996). Studies researching the effect of the protein content of various snacks are more comparable in terms of protein content to that which was provided by the treatments in the present study (Douglas, Ortinau et al. 2012, Ortinau, Culp et al. 2013, Ortinau, Hoertel et al. 2014). In these snack studies, the high protein treatments contained 14g protein (Ortinau, Culp et al. 2013, Ortinau, Hoertel et al. 2014) and 24g protein (Douglas, Ortinau et al. 2012) per treatment which is similar to the 20g protein provided by the mean intake of the dairy treatment in the present study. In one of the snack studies, subsequent intake was measured at a meal by requested of participants (Douglas, Ortinau et al. 2012). Meal request times of 152, 158, and 178 minutes for low protein, moderate protein, and high protein snacks suggest that the present study’s 120 minute time frame between the
treatment and pizza meals may not have been sufficient time for participants to become sufficiently hungry for a subsequent meal (Douglas, Ortinau et al. 2012).

A study that specifically examined the effects of locust bean gum, one of the fibre containing ingredients in the non-dairy treatment used in this study, found 6g of locust bean gum resulted in a reduction in gastric emptying of healthy men and women (Darwiche, Bjorgell et al. 2003). This is comparable to the amount of fibre provided from the average non-dairy treatment consumption in this study, however it is important to remember that this includes both the fibre from the non-dairy cultured coconut milk and the granola, therefore the amount of fibre from locust bean gum in this study would be less than that provided in the Darwiche study. Additionally the soluble fibre found in oatmeal that is associated with increased satiety (Rebello, Johnson et al. 2013) was present in both treatments from the granola (5g fibre/54g granola). This, in conjunction with the effects of the dairy protein, may help to explain why significant differences in ad libitum food intake or subjective measures of satiety were not observed between the two treatments.

No session effect of the pizza meals was observed to indicate participant fatigue with receiving the same meal at all three sessions. This may have been avoided as sessions were scheduled at least one week apart and the three sessions were completed over, at minimum, a two month time frame to accommodate menstrual cycle timing of the participants.

No statistically significant differences in pleasantness, taste, texture, or pizza pleasantness scores between the dairy and non-dairy treatments were observed suggesting that the caloric treatments were consumed based on satiation rather than participant taste preferences.
The statistically significant, strong, correlations between VAS appetite scores and food intake suggests that the VAS scales used were valid and accurate measures of hunger and appetite. As would be expected, there was a significant, strong, negative correlation observed between treatment intake and average desire to eat scores indicating that the treatment intake did reduce participant desires to eat. Likewise, mean fullness and hunger scores were both also significant, strong and negatively correlated to treatment intake. While still significant, the correlation between desire to eat, hunger, and potential food consumption scores and pizza intake was moderately positive, further suggesting that participants may not have yet been ready for a full meal at the two hour point when the pizza meal was provided. Similarly, scores of average appetite were significantly, strongly and negatively correlated with treatment intake but only moderately positively correlated with pizza intake.

Potential limitations of this study include the population studied. Many of the individuals who took part in the study were recruited from the department of Applied Human Nutrition. Given the nature of the questions on the restrictive eating questionnaire, it was thought that these students may receive falsely high scores on the questionnaire due to the nature of their studies rather than as a true reflection of their eating habits. For example, questions like those that ask if the individual “gives too much time and thought to food” and if they are “conscious of what you are eating” were thought to potentially score higher for students in a nutrition program regardless of their eating habits. Thus, to prevent the unnecessary exclusion of nutrition students due to falsely high scores, individuals who scored higher on the restrictive eating
questionnaire were still permitted to participate in the study. This may have permitted individuals who actually do have restrictive eating habits to participate in the study, skewing the results. However, only eight of the participants who completed the study had scores that would potentially indicate restrictive eating habits.

While the two caloric treatments were selected to be as similar as possible in terms of palatability, taste, and nutrient composition, the nature of using commercially available products limited the ability to fully achieve that. Thus, the ability to correlate the results of the study to the dairy vs. non-dairy attributes of the caloric treatments is limited. The products were minimally augmented so they would provide similar amounts of available carbohydrates as that was thought to be a significant factor contributing to blood glucose levels and corresponding insulin response (Augustin, Kendall et al. 2015). However, other attributes of the products may have influenced food intake. For example, the non-dairy product contained 5.6 g fibre/100g. As fibre is known to contribute to feelings of fullness, this may have contributed to increased satiety and satiation from the non-dairy product as compared to the fibre free dairy product. Similarly, the dairy treatment contained 9.2 g protein/100g which is also known to influence satiety and satiation. Future studies would benefit from comparing products that do not significantly differ in different compositional attributes that may influence the characteristics being studied. I.e. both products should contain protein or fibre to be able to more accurately discern the role of the dairy vs. non-dairy base on the measures of interest.

The participants in this study ate less at the pizza meal than was anticipated. Based on the population studied and those results, it is possible that perceptions of appropriate amounts of
foods, particularly pizza, consumed may have played a role in intake in addition to actual feelings of hunger, satiety, and satiation.
Chapter 7: Significance and Implications for Future Research

The results of this study demonstrate that the dairy cultured product, consumed with granola cereal, resulted in a reduced postprandial blood glucose response and an increased blood insulin response compared to the non-dairy cultured product and granola. This data, and the outcomes of related research, provide beneficial intake information that can be used by individuals and practitioners in making dietary choices related to blood glucose control. These results suggest that for individuals looking to make dietary choices that result in a reduced postprandial glycemic response, the dairy cultured product offers greater benefits in that regard compared to non-dairy cultured product.

This study was designed and completed in compliance with the Health Canada satiety claims document and therefore used water as an energy-free control, however future studies may benefit from using a caloric control (ie. granola alone) to allow for a more complete comparison of the dairy and non-dairy products used.

Future research should be expanded to include males to determine if the blood glucose and insulin response to dairy vs non-dairy cultured products is similar between the sexes and to determine if there are differences in terms of subjective measures of appetite and food intake. Similarly, given current research suggesting differences in hormonal responses to food and eating behaviours between normal weight and overweight and obese individuals, future research would benefit from the inclusion of overweight and obese individuals. As it stands, the findings of this research can only be extrapolated to the healthy female population that was studied.
Chapter 8: Conclusion

Based on the results of this study, the breakfast meal formulated with the dairy fermented product resulted in a reduced postprandial glycemic response without an increase in subsequent energy intake as compared to a meal formulated with a non-dairy fermented product. This suggests that dairy fermented products may offer benefit as a functional breakfast for improved blood glucose control.
References:


McCrickerd, K., et al. (2014). "Does modifying the thick texture and creamy flavour of a drink change portion size selection and intake?" Appetite 73: 114-120.


Appendices

Appendix A
Telephone Screening Questionnaire Part 1 of 3

Methods

Purpose:
This questionnaire will be administered by the researcher for the purpose of recruitment of participants.

Methods:

The researcher will ask to speak with the indicated potential participant. If the researcher is unable to reach the participant, they will leave a message and try again the following day. If they do not hear back they will try one more time and if they are unsuccessful again they will remove the participant from the sample pool, assuming that the participant is not interested in the study.
Telephone Screening Questionnaire Part 2 of 3

Participant Identity

Name: ___________________________________________ ID assigned: __________________

Age: ________________ years

Date of Birth: (d/m/y)_____________________________________

TO BE KEPT SEPARATELY
Telephone Screening Questionnaire: part 3 of 3

*Please print or circle the answer*

ID: ____________________

Age: ____________________

Height: _____________________________ cm

Weight: __________________________ kg

Have you lost or gained weight recently? Yes / No

Do you follow a special diet? Yes / No

Do you have any food allergies or food sensitivities? Yes / No

(If yes please explain: _____________________________________________________)

Health Problems? Yes / No (If yes please explain: _____________________________________)

Medications?: Yes / No (If yes please explain: _____________________________________)

Do you have any learning difficulties/problems? Yes / No

(If yes please explain: ______________________________________________________)

Do you have any behavioral or emotional problems? Yes / No

(If yes please explain: ______________________________________________________)

Invite for screening? Yes / No

Appointment scheduled for: (date and time)

_______________________________________________________________________

Investigator/Date screened: _____________________________________________________________________
Appendix B

Department of Applied Human Nutrition,
166 Bedford Highway, Halifax, NS Canada B3M 2J6

Information Sheet and Consent Form

The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

Information Sheet and Consent Form

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**Introduction:**

You are invited to participate in the research study listed above. This form provides you with information about the study so you can make an informed decision about if you would like to participate. It will enable you to understand the purpose of the study, the risks and benefits of participating, and what you will be asked to do should you choose to participate. We will keep you informed of any new information that may influence your willingness to continue to participate in the study. A member of the research team will be available to answer any questions you may have. You may decide not to participate and you may withdraw from the study at any time. Your participation in the study is entirely voluntary.

**Funding Source:**

Funding for this project is provided by the Dairy Farmers of Canada and Agriculture and Agri-Food Canada. There are no conflicts of interest between the investigators and the sponsors.

**Background and Purpose of Research:**

Breakfast cereals are traditionally consumed with dairy products including milk and fermented milk products such as yogurt. Yogurt consumption continues to grow but many consumers are also shifting towards dairy-free alternatives that are perceived to be a healthier option to dairy products.

The purpose of this study is to determine the effect of dairy and non-dairy cultured products used as a carrier for breakfast granola cereal on blood glucose control, appetite and food intake.

This study will have 25 female participants.

**Invitation to Participate:**

You are being invited to take part in this study. If you chose to take part and meet eligibility criteria, you will be asked to eat a treatment of granola cereal mixed with Greek yogurt, granola cereal mixed with coconut yogurt, or plain water three times (three sessions) with each session one month apart. At each session, your blood will be collected by a professional phlebotomist or registered nurse and your appetite will be measured using simple paper scales after eating the treatment. Each session will take up to three hours of your time.

**Eligibility:**

To participate in this study you must be considered healthy, which for this study is defined as not being overweight or obese and not having any diseases. You must also be between the ages of 19 and 35. You must be a nonsmoker and you cannot be taking any medications. You will not be able to participate if you have allergies to any food or if you usually skip breakfast.

The study will take place in the Department of Applied Human Nutrition, Rm 365 Evaristus Building, 166 Bedford HWY, Halifax, NS.

**Procedure:**

To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured. Participants will be asked to attend experimental sessions during the same time of their menstrual cycle (i.e.
one experimental session once a month) and therefore will be asked to provide information about their menstrual cycle at the screening session.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for three sessions over three months.

You will be asked to arrive at the Evaristus Building between 8:00 a.m. and 10:00 a.m. but your arrival time should be the same for each experimental session. You will be asked to stick to your normal routine, including exercise and to eat a similar meal the night before each session and then stay fasted for at least 12 hours until you arrive to the laboratory the next morning. You can drink water up to one hour before arriving at the session. After arrival to the lab, you will be asked to complete a questionnaire about your recent food intake and if any medication was taken. You will then be asked to have a blood sample taken to measure your fasting blood glucose levels. A professional phlebotomist (blood collector) or nurse will insert a tiny cannula (tubing) into your arm vein so we will not need to poke you every time we need to take a blood sample. This cannula will stay in your vein for two hours. At each session you will be asked to eat a treatment and to complete questionnaires at the times outlined in the table below. The treatment will consist either of (1) granola mixed with Greek yogurt or (2) granola mixed with coconut yogurt or (3) spring water. These treatments will be provided in a random order over three sessions. We will serve the treatment to you and will ask to eat it until you feel comfortably full. Then you will be asked to provide a further 6 blood samples (for a total of 7) over the next 2 hours, at 15, 30, 45, 60, 90, and 120 minutes. In two hours, you will be asked to eat a lunch meal (pizza) until you feel comfortably full. You will be asked to fill out visual analogue scale (VAS) questionnaires measuring your appetite and physical comfort as well as the palatability (pleasantness) of the treatment. Water will be provided with the treatments and lunch meals.

Each session will last up to three hours.

An Example of a Potential Time and Activity Schedule for Each Session:

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:45</td>
<td>Arrive at the laboratory</td>
</tr>
<tr>
<td>8:50</td>
<td>Fill in Sleep, Stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>9:00 - 9:10</td>
<td>Eat the treatment (0 min).</td>
</tr>
<tr>
<td>9:10 – 11:10</td>
<td>Fill out VAS questionnaires at 15, 30, 45, 60, 90 and 120 min</td>
</tr>
<tr>
<td>11:10 – 11:30</td>
<td>Eat the lunch meal until you feel comfortably full.</td>
</tr>
</tbody>
</table>

VAS= Visual analogue scale

Voluntary Participation and Early Withdrawal:

Participation in this study is voluntary. You may choose to stop being in the study at any time without any negative consequences.
Risks:
All blood samples gathered will be done so by a trained Nurse or Phlebotomist, and all samples will be collected using aseptic techniques in a hygienic environment. Each sample will be 8.5 ml, for a total of less than 70 ml, far less than that taken when donating blood. All of the foods that you will be asked to consume will be prepared using practices to ensure food safety. Therefore, your risk of developing a food borne illness from participation in this study is very minimal.

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

There is always a possibility that you may become ill following consumption of food, but this is very unlikely. All treatments are freshly prepared at the time of your session.

Benefits:
You will not benefit directly from taking part in this study. However the study results will advance nutritional science and may lead to practical dietetic recommendations.

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. If you withdraw from the study, your consent form and any other paperwork associated with your ID number will be destroyed.

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, no information about individuals will be reported.

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 $30 per experimental session and $5 per session for travel. Payment will be in the form of a cheque and will be paid for each session.

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 Participants:

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 questions about how this study is being conducted and wish to speak with someone not involved in the study, you may contact the Chair of the University Research Ethics Board (UREB) c/o MSVU Research Office, at 457-6350 or via e-mail at research@msvu.ca.

The ethical components of this research study have been reviewed by the University Research Ethics Board and found to be in compliance with Mount Saint Vincent University's Research Ethics Policy.

Dissemination of findings:

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

Copy of informed consent for participant:

You will be 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
Appendix C
Baseline Information Questionnaire

The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

Please type

NAME: ________________________________________________________________

ADDRESS:

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

PHONE #: (________)_______________ E-MAIL: ____________________________

ID assigned: ________________

To be kept separately from part 2 and other study forms
Appendix C (part 2)
Baseline Information Questionnaire

The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

(NOTE: After you are recruited for the study, you will be assigned an ID# which will be used on your forms and data throughout the study.)

To be completed by Research Personnel:

ID: ______________________

AGE: _____ HEIGHT: _______WEIGHT: _______BMI: ____________

To be completed by Participant:

Participation in Athletics/Exercise:

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>HOW OFTEN?</th>
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Do you usually eat breakfast?  
☐ YES  ☐ NO

If YES, what do you usually eat?
______________________________________________________________________________
______________________________________________________________________________

Health Status:

Do you have diabetes?  
☐ YES  ☐ NO

Do you have any other major disease or condition?  
☐ YES  ☐ NO

If YES, please specify:
______________________________________________________________________________
______________________________________________________________________________

Are you taking any medications?  
☐ YES  ☐ NO

If YES, please specify:
______________________________________________________________________________

Do you have reactions to any foods?  
☐ YES  ☐ NO

If YES, please specify:
______________________________________________________________________________

Are you on a special diet?  
☐ YES  ☐ NO

If YES, please specify:
______________________________________________________________________________

Have you recently lost or gained weight?  
☐ YES  ☐ NO

If YES, please specify:
______________________________________________________________________________

Do you smoke?  
☐ YES  ☐ NO

How many alcoholic beverages do you consume per day? _________ Per week? _________
Appendix D
Eating Habits Questionnaire

The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

Choose the appropriate answer to best describe your personal situation.

1. How often are you dieting? (Scored 0-4)
   _____ Never; _____ rarely; _____ sometimes; _____ often; _____ always

2. What is the maximum amount of weight (in pounds) that you have ever lost within one month? (Scored 0-4)
   _____ 0 - 4; _____ 5 - 9; _____ 10 – 14; _____ 15 – 19; _____ 20+

3. What is your maximum weight gain within one week? (Scored 0-4)
   _____ 0 – 1; _____ 1.1 – 2; _____ 2.1 – 3; _____ 3.1 – 5; _____ 5.1+

4. In a typical week, how much does your weight fluctuate? (Scored 0-4)
   _____ 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? (Scored 0-3)
   _____ Not at all; _____ slightly; _____ moderately; _____ very much

6. Do you eat sensibly in front of others and splurge alone? (Scored 0-3)
   _____ Never; _____ rarely; _____ often; _____ always

7. Do you give too much time and thought to food? (Scored 0-3)
   _____ Never; _____ rarely; _____ often; _____ always

8. Do you have feelings of guilt after overeating? (Scored 0-3)
   _____ Never; _____ rarely; _____ often; _____ always

9. How conscious are you of what you are eating? (Scored 0-3)
   _____ Not at all; _____ slightly; _____ moderately; _____ extremely

10. How many pounds over your desired weight were you at your maximum weight? (Scored 0-4)
    _____ 0-1; _____ 2 – 5; _____ 6 - 10; _____ 11-20; _____ 21+
Appendix D(i): Rigid Control Dietary Restraint Form

The Effect of Dairy and Non-Dairy Cultured Products Added to Breakfast Cereals on Satiety, Blood Glucose and Food Intake

Flexible Control (FC12) - Please circle that response that best suits you.

1. When I have eaten my quota of calories, I am usually good about not eating any more.
   
   True  –  False

2. I deliberately take small helpings as a means of weight control.
   
   True  –  False

3. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.
   
   True  –  False

4. I consciously hold back at meals in order not to gain weight.
   
   True  –  False

5. I pay a great deal of attention to changes in my figure.
   
   True  –  False

6. How conscious are you of what you are eating?
   
   Not at all – Slightly – Moderately – Extremely

7. How likely are you to consciously eat less than you want?
   
   Unlikely – Slightly unlikely – Moderately likely – Very likely

8. If I eat a little bit more on one day, I make up for it the next day.
   
   True  –  False

9. I pay attention to my figure, but I still enjoy a variety of foods.
   
   True  –  False

10. I prefer light foods that are not fattening.
   
    True  –  False

11. If I eat a little bit more during one meal, I make up for it at the next meal.
    
    True  –  False

12. Do you deliberately restrict your intake during meals even though you would like to eat more?
Always – Often – Rarely – Never

Rigid Control (RC16) - Please circle that response that best suits you.

1. I have a pretty good idea of the number of calories in common food.  
True – False

2. I count calories as a conscious means of controlling my weight.  
True – False

3. How often are you dieting in a conscious effort to control your weight?  
Rarely – Sometimes – Usually – Always

4. Would a weight fluctuation of 5 lb affect the way you live your life?  
Not at all – Slightly – Moderately – Very much

5. Do feelings of guilt about overeating help you to control your food intake?  
Never – Rarely – Often – Always

6. How frequently do you avoid “stocking up” on tempting foods?  
Almost never – Seldom – Usually – Almost always

7. How likely are you to shop for low calorie foods?  
Unlikely – Slightly unlikely – Moderately likely – Very likely

8. I eat diet foods, even if they do not taste very good.  
True – False

9. A diet would be too boring a way for me to lose weight.  
True – False

10. I would rather skip a meal than stop eating in the middle of one.  
True – False

11. I alternate between times when I diet strictly and times when I don’t pay much attention to what and how much I eat.  
True – False

12. Sometimes I skip meals to avoid gaining weight.  
True – False

13. I avoid some foods on principle even though I like them.  
True – False
14. I try to stick to a plan when I lose weight.

True  –  False

15. Without a diet plan I wouldn’t know how to control my weight.

True  –  False

16. Quick success is most important for me during a diet.

True  –  False
Appendix E
Visual Analogue Scales
Palatability: Treatment

This question relates to the palatability of the beverage/food you just consumed. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present findings.

1. How pleasant have you found the beverage/food?

   NOT ___________________________ VERY pleasant
   at all pleasant

2. How tasty have you found the treatment?

   NOT ___________________________ VERY tasty
   at all tasty

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

   NOT ___________________________ VERY much
   at all much
These questions relate to your “motivation to eat” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

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

   VERY ________________________________ VERY strong
   weak

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

   NOT ________________________________ As hungry
   hungry as I have ever felt
   at all

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

   NOT ________________________________ VERY full
   full at all

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

   NOTHING ________________________________ A LARGE
   at all amount

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

   NOT ________________________________ As thirsty
   thirsty as I have ever felt
   at all
Appendix G
Visual Analogue Scales
Energy and Fatigue

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

1. How energetic do you feel right now?

   NOT ________________________________           VERY energetic
   at all

2. How tired do you feel right now?

   NOT ________________________________           VERY tired
   at all
Appendix H
Visual Analogue Scales
Physical Comfort

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

1. **Do you feel nauseous?**

   NOT (__________________________ )        VERY (__________________________ )
   at all                           much

2. **Does your stomach hurt?**

   NOT (__________________________ )        VERY (__________________________ )
   at all                           much

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

   NOT (__________________________ )        VERY (__________________________ )
   well                             well
   at all

4. **Do you feel like you have gas?**

   NOT (__________________________ )        VERY (__________________________ )
   at all                           much

5. **Do you feel like you have diarrhea?**

   NOT (__________________________ )        VERY (__________________________ )
   at all                           much
Appendix I
Visual Analogue Scales
Palatability: Lunch meal

This question relates to the palatability of the beverage/food you just consumed. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present findings.

How pleasant have you found the food?

NOT at all pleasant .......................... .......................... VERY pleasant