The Effect of Dairy and Non-dairy Snack Products on Glycemic Regulation in Normal Weight Children

By

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Increased snacking in children is associated with higher energy and sugar intake, known risk factors for obesity and diabetes. The objective of this study was to determine the effect of dairy and non-dairy snacks on glycaemia in children. Methods: In a repeated measures crossover design, normal weight (5th-85th BMI percentile) children (n =11, 5 boys and 6 girls; age: 9-14 y), were randomly assigned to consume one of two treatments: Greek yogurt (171 kcal) and mini sandwich type cookies (175 kcal). Both treatments contained 25 g of available carbohydrates. After an overnight fast, children consumed a standardized breakfast in the morning, two hours before arriving at the lab. Venous blood samples were collected for glucose and insulin at 0 min (immediately before the treatment), and at 30, 60, 90 and 120 min. Results: There was an effect of treatment, time and a time by treatment interaction ($P<0.0001$) on blood glucose and insulin over 120 min. The Greek yogurt treatment resulted in lower glycaemic and higher insulin responses compared to the cookies treatment ($P<0.0001$). This effect can be explained by the higher content of protein in the Greek yogurt treatment (17 g) compared to the cookies treatment (1.3 g). Conclusion: The available carbohydrate content is not the only predictor of postprandial glycemia but rather the macronutrient composition of a snack predetermines its glycaemic response in children.

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Abbreviations

A	
AUC	Area under the curve
B	
BCAA	Branched Chain Amino Acids
BD	Becton Dickson Canada Inc.
BG	Blood Glucose
BMI	Body Mass Index
C	
CCHS	Canadian Community Health Survey
CCfV	Canadian Center for Vaccinology
CCK	Cholecystokinin
CDC	Center for Disease Control
CSFII77	Continuing Survey of Food Intake by Individuals 1977-1979
CSFII89	Continuing Survey of Food Intake by Individuals 1989-1991
CSFII98	Continuing Survey of Food Intake by Individuals 1994-1996, 1998
CVD	Cardiovascular Disease
F	
FI	Food Intake
G	
GI	Glycemic index
G:I	Glucose to Insulin Ratio
GIP	Gastric Inhibitory Peptide or Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1
I	
IR	Insulin Resistance
K	
kJ	Kilojoule
M	
MetS	Metabolic Syndrome
METs	Metabolic Equivalents
M.F.	Milk Fat
N	
NHANES III	National Health and Nutrition Examination Survey 1988-1994
NLSCY	National Longitudinal Survey of Children and Youth
S	
SQ	Satiety Quotient
V	
VAS	Visual Analogue Scale

Chapter 1

Introduction

Carbohydrate metabolism is instrumental in glycemic control and food intake regulation due to the high turn-over, limited storage, immediate and tight regulation, and the critical role it plays as a fuel source for the brain (1). Once carbohydrates are digested, they are absorbed from the intestine into the blood stream, which produces a rise in blood glucose concentrations. Postprandial hyperglycemia (and associated insulinemia and lipidemia) has been associated with the etiology of chronic metabolic diseases such as type 2 diabetes (T2DM) and cardiovascular disease (2). A large portion of research has focused on children and T2DM as the increase in this population has grown over the last decade concurrently with a rise in obesity rates (3). Children classified as obese are at a higher risk for increased blood pressure, abnormalities in serum lipids levels, and increased rates of insulin resistance (a precursor to the development of diabetes) than normal weight children (4). Obesity is the most significant risk factor for insulin resistance (IR) in adolescents (5).

In addition to T2DM, rates of overweight and obesity have increased steadily in the past few decades so much so it has been termed an epidemic (6-9). Population data from Canada shows an increase in overweight and obesity rates among children 2-17 year olds (4). This is of particular importance as overweight adolescents have a propensity towards adult obesity when compared to normal weight adolescents (10-12), making children and adolescents a focus for the obesity pandemic.

Snacking is a large part of the North American diet, data from population studies in the U.S. show that the prevalence of snacking has increased approximately by 20% from 1977 to 1996 for children of all ages (13). Along with the increase in snacking in children there has also been a shift in the consumption of key food groups. There is an increase in total energy intake for 2 to 18 year olds with respect to energy from salty snacks, pizza, and soft drinks. Concurrently, the same age group shows a decrease in milk (low and medium fat) consumption as a snack (14). Specific to Canada a small study looking at food consumption in 8 to 10 year olds showed that 58.4% of children are not meeting the recommended amount of milk products for Canada's Food Guide (3-4 servings) (15).

Dairy products are of interest in nutritional research due to the specific properties of their constituents (16). Frequent consumption of dairy products is associated with lower

cardiovascular disease, lower prevalence of insulin resistance (17), lower risk of type 2 diabetes (18), and may have a potential role in the acceleration of weight loss (19). In terms of children, there is very little known about the effect of snacks as well as milk products on glycemic response. To determine a causal relationship between glycemic regulation in children and snacking, short-term experimental studies are needed to investigate the acute blood glucose and insulin response to the ingestion of dairy and non-dairy foods.

Chapter 2

Literature Review

2.1 Impaired Glycemic Control & Metabolic Disorders in Children

Glycemic regulation in children is an area of focus in appetite and obesity research as carbohydrates make up the main source of energy in the diet (2). Elevated post-prandial hyperglycemia has been associated with the development of CVD and T2DM (2). Blood glucose concentrations are typically tightly controlled through metabolic pathways however, when the body is unable to maintain the concentrations, due to insulin resistance, diabetes and other metabolic disorders develop.

Prediabetes is a condition that exists prior to the development of diabetes and puts individuals at higher risk of developing diabetes. Two important factors for pre-diabetes are impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). The diagnostic criteria developed by the Canadian Diabetes Association defines IFG as a fasting plasma glucose (FPG) value (following an eight hour fast) from 6.1 – 6.9mmol/L (20). Impaired glucose tolerance is defined as two hour plasma glucose of 7.8-11.0 following an 75g oral glucose tolerance test (20). Lifestyle interventions have shown to be the most beneficial to delaying or preventing the onset of diabetes in people with IGT (21).

2.1.1 Diabetes

Diabetes is a metabolic disturbance that is characterized by hyperglycemia (high blood glucose) and is associated with vascular complications (22). Typically, once hyperglycemia develops, there is already a reduction in insulin sensitivity and beta-cell function (23). Type I diabetes occurs when the pancreas is not able to make or secrete insulin resulting in hyperglycemia. Type II diabetes occurs when the insulin the body makes is insufficient or resistant to the target tissues (liver and muscles) (22).

Type II diabetes is a chronic disease that is growing rapidly and will reach pandemic proportions by 2030 (24). A large portion of research has focused on children and T2DM as the increase in this population has grown over the past few years concurrently with a rise in obesity rates (3). The incidence rate of T2DM in Canadian children under the age of 18 is 1.54 cases per 100,000 children and the average age is 13.7 years old (3). Similar to adults, children who have T2DM also present with obesity, insulin-resistance, and beta-cell dysfunction (23). This leads to

hyperinsulinemia (high levels of insulin in the blood), beta cell failure, and eventually hyperglycemia as the body is unable to produce sufficient amount of insulin (25). There is a concern that the increasing rates of children with T2DM will have a shorter lifespan than that of their parents generation (5). Children who develop T2DM typically develop additional metabolic abnormalities as a result of insulin resistance and these health risks have been grouped together and are known as Metabolic Syndrome.

2.1.2 Metabolic Syndrome

Insulin resistance and obesity are risk factors for serious health conditions such as heart disease, hypertension, and T2DM (8, 26, 27). These health disturbances have been researched extensively and are clustered into what is known as Metabolic Syndrome (MetS). Insulin resistance (27) as well as obesity (22) are both key to the development of MetS. The Canadian Diabetes Association, through the recommendation of Alberti and colleagues (28), defines MetS as the presence of three or more of following criteria: elevated waist circumference (≥ 103 cm for men and ≥ 88cm for women), elevated triglycerides (≥1.7 mmol/L), elevated blood pressure (≥130/85mm Hg), elevated fasting plasma glucose (≥5.6 mmol/L), and reduced HDL cholesterol (<1.0 mmol/L in men and <1.3 mmol/L in women) (20). Individuals with MetS are at risk of developing cardiovascular disease (CVD) and diabetes. Evidence suggests that aggressive treatment for hyperglycemia as well as cardiovascular risk factors can reduce the morbidity and mortality associated with CVD (21).

Originally MetS was examined in the adult population however the presence of these abnormalities in children is on the rise (27, 29, 30). Defining metabolic syndrome is difficult in adolescents as they are going through a period of growth and development (22, 27). When defining insulin resistance in children, the challenge is the associated insulin-resistance state that occurs as a result of normal pubertal development (22). Management of MetS in children is difficult as the use of pharmacological treatment is rarely recommended. Most often management requires dietary intervention as well as regular physical activity to help reduce abdominal adiposity (25, 30).

2.2 Food Consumption Patterns in Children

2.2.1 Background

How people access their food is an environmental factor that plays a role in what foods individuals consume. Traditional food sources (i.e. food that can be categorized into a food group) are declining while less traditional food sources, typically more energy dense, are increasing (31). Some of the shifts away from traditional food sources occur through food consumed away from home, for example at fast-food restaurants (9, 14). In 1970, approximately 33% of American's food dollars were spent on food eaten away from home, in 2001 the rate increased to 47% (32).

Low nutrient dense (LND) foods have also been an area of focus for researchers. Typically, LND foods consist of foods that have added fat and sweeteners that are energy dense and nutrient poor. Research examining the consumption of LND foods, from National Health and Nutrition Examination Survey III, 1988-1994, (NHANES III, 1988-1994) shows that these types of foods also accompany higher energy intakes in children (33). Also nationally representative surveys shows that the intake of specific foods; salty snacks, soft drinks, and pizza, have increased from 1977-1996 while in the same time period there has been a decrease in milk products (14).

Numerous studies indicate that snack consumption is on rise and is contributing to overall energy intake (13). Snacking has increased from 77% to 91% of 2-18 year olds which is based on population food intake data collected in the U.S. from 1977 to 1996 (13). The specific increases seen in snacks have typically been in low nutrient and high caloric value foods. The wide availability of energy dense, nutrient poor, snacks (34) is potentially contributing to the increase in snacking occasions, which may also play a role in glycemic control and eventually lead to weight gain.

Snacks that are typically consumed have a decreased satiating (feeling of fullness) effect (35) but potentially contribute to a significant amount of calories leading to a positive energy balance and weight gain. Researchers began to look at the shift in key food groups, which shows a decrease in milk consumption (14, 15, 31). Dairy consumption is a popular area in research as there is a negative association between dairy consumption and body weight (36, 37). More recently the Third National Health and Nutrition Examination Survey has shown that dairy food consumption in adolescents is inversely associated with abdominal obesity (38). The increase in snacking paired with decrease milk consumption and growing rates of childhood obesity and T2DM have highlighted the importance of conducting further research to understand how

snacking and specifically which snacks affect glycemic response and food intake in the short term.

2.2.2 Snacking Behavior

Currently, there is no universally accepted definition or classification for snacks vs. meals (13, 39-41). There are many methods to define "snack"; some are based temporally, others are based on specific composition or caloric allotment, and also a variety of hybrid definitions are used (39, 40, 42). Epidemiological studies for food intake data in the U.S. define snacks through a self designated definition (determined by the consumers) (39). This self-reported data classified the food item as snack vs. non-snack by showing pictorial representation of typical meals and snacks, time taken to eat the food item, and the food item consumed (13).

Snacking affects overall caloric intake (42) and over time can lead to a positive energy balance resulting in weight gain (39). Western diets are moving toward more frequent eating occasions potentially due to the wide availability of foods as well as an increase in foods eaten away from home (43). Food intake including snacking can be mediated through homeostatic factors (e.g., glucostatic theory) as well as non-homeostatic factors. Non-homeostatic factors that influence eating are habit, time of day, stress, convenience, and various social interactions (44).

Data from the National Food Consumption Survey 1977-79 (NFCS77), Continuing Survey of Food Intake by Individuals 1989-91 (CSFII89) and 1994-96,1998 (CSFII98) showed that while the portion of snacks remained the same, the quantity of snacking occasions increased. The major snacking categories for 2-18 year olds in the U.S. is derived from desserts and sweetened beverages followed by salty snacks and candy (35). Data from the Canadian Community Health Survey Cycle 2.2 Nutrition (2004) indicates 63% of children and adolescents consume an afterschool snack and the majority of intake comes from energy-dense and nutrient poor snacks (e.g. cookies and cereal bars) (45). Additionally, a high intake of after-school snacks seems to be linked to higher overall energy intake.

The amount of snacks per day significantly increased ($p < 0.01$) from 1.73 to 2.29 for 2 - 5 year olds, from 1.56 to 1.99 for 6-11 year olds, 1.6 to 1.97 for 12-18 year olds (13, 35, 39). Population data from Canada indicates overweight and obesity rates have increased in the same time period (4). The population data does not indicate a causal relationship between snacking and obesity; however, this rise has sparked further research in understanding the relationship between snacking (and specifically types of snacks) and obesity.

2.2.3 Dairy Consumption

Results from the NHANES 1999-2002 shows that intakes of dairy are inadequate for children ages 4-18 (46). Also nationally representative data from the US has shown a decrease in overall energy from milk and dairy consumption for ages 2-18 year olds between 1977- 2006 (35). Canada's food guide recommends 3-4 servings of milk and alternatives for the ages 9-14 (47). One serving is equivalent to one cup of milk, 175g of yogurt, or 50g of cheese. Specific to Canada, over 50% of children ages 8 -16 years are not meeting the recommended amount of dairy products as recommended by Canada's Food Guide and only 21% (15, 48).

2.3 Physiological Mechanisms of Blood Glucose Control and Food Intake Regulation

2.3.1 Glycemic Regulation

Energy homeostasis is achieved through the body's regulation of nutrient storage in specific tissues (fat in adipose tissue and glycogen in liver and muscles) and nutrient levels in the blood (blood glucose) (49). Glycemic regulation is important to maintaining homeostasis as carbohydrates are the main source of energy for the human diet consisting of around 40 -80% of total energy intake (50). Carbohydrate (CHO) metabolism is instrumental in food intake regulation due to the high turn-over, limited storage, and immediate and tight regulation (1). Carbohydrates that are digestible are absorbed from the intestine into the blood stream, which produces a rise in blood glucose concentrations that exceeds the fasting level. The pancreas is signaled to expect the rise in blood glucose through glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) which accelerates a rise in beta cell release of insulin (50). The glucose in the blood is then transported to target tissues and entrance to those tissues is facilitated by insulin which decreases the glucose concentrations back to fasting levels (51). Insulin suppresses hepatic glucose production and facilitates the utilization and storage of glucose by the liver and other tissues (22).

Glycemic response is measured by the rise and fall of blood glucose and the period of time it takes for the glucose concentration to return to fasting levels. One way of measuring glycemic response to certain carbohydrates in food is through the use of the glycemic index (GI). GI is the response of blood glucose concentration to an individual given a test food (usually containing 50g CHO) over a set period of time that can be compared to a standard food consumed by the individual (usually glucose which has a GI of 100) (51). However GI also has

limitations as it does not account for processing, food form, the presence of fat or protein (52), and it has poor accuracy related to between subject variability (53).

2.3.2 Glycemic Regulation and Physiological Control of Food

Sugars have been an area of focus in determining the key factors in childhood obesity. There is a hypothesis that glycemic response to high GI foods stimulates appetite and leads to excess energy intake. This occurs through an increase in glucose concentration in the blood and the body's response by secreting insulin to return to a fasting level. However, high glycemic foods can result in a large insulin spike which drives glucose below fasting (hypoglycemic) levels which results in hunger and potentially excessive food intake (52).

Jean Mayer investigated the interaction between glucose concentrations and satiety. His glucostatic theory of appetite control states that as blood glucose concentrations rise, there is also an increase in satiety, and when blood glucose concentrations fall, there is an opposite effect on satiety (54, 55). The theory states that changes in blood glucose concentrations are detected by the hypothalamus and food is ingested when cell glucose utilization is unable to meet the demand for tissues and organs that require glucose in the body (52). Prior to meal initiation, there is a transient drop in blood glucose concentrations (52, 56, 57) however the cause of this event is unknown. A study by Melanson et al. examined the impact of CHO, fat, and aspartame on appetite and blood glucose in time-blinded males. The results showed that the high fat drink led to a later meal initiation than the carbohydrate drink. However, the total food intake did not differ between the two, this study highlighted the important role that blood glucose plays on physiological regulation of meal pattern (58). Glucose metabolism is also regulated by insulin-independent mechanisms for example; the rate of gastric emptying is a result of the presence of certain gut hormones (e.g. GLP-1) that are integral to the metabolism of carbohydrates. The types of hormones that are related to glucose homeostasis are also closely related to adiposity control.

The regulation of body adiposity is achieved by two signals: long-term adiposity signals (e.g. insulin and leptin) and short-term satiation signals (e.g. CCK) (59). Hormones that are important in the control of body weight and appetite are cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), ghrelin, insulin, and leptin. CCK is a peptide secreted in the duodenum due to a response to lipids or protein. The ingested proteins or lipids then activate receptors on sensory nerves in the duodenum which sends a message to the brain that contributes to satiation

(49). CCK rises during a meal and after a meal to suppress feelings of hunger. GLP-1 is known as a satiety peptide, and is secreted in response to nutrients (such as carbohydrate and fat). GLP-1 plays a role in metabolism by stimulating insulin secretion and inhibiting glucagon release (49). Ghrelin is secreted in the stomach and plays a role in weight management as it stimulates food intake (49) and is known as the only circulating orexigen (60, 61). Ghrelin is also known as a hunger signal. The levels of ghrelin rise during a meal and fall about an hour after food intake (62).

Hormones insulin and leptin play an important role in long-term control of energy stores in the body and basal circulating levels of both hormones are secreted in direct proportion to fat stores (49). With respect to insulin elevated fasting insulinemia due to insulin resistance (a precursor for diabetes) is implicated in the development of cardiovascular disease in adults (63) and children (64). In terms of leptin, when a deficiency occurs it is known to promote hunger as well as reduce energy expenditure (62). When considering starvation, the body becomes less sensitive to leptin and insulin levels, therefore a greater amount of food is needed to produce a sufficient satiation signal to stop eating (49).

2.4 The Role of Dairy Products and Ingredients in Blood Glucose Control

Dairy consumption is an area of focus for researchers examining obesity as previous studies have shown a relationship between dairy intake and healthy body weights (38, 65). Dairy has favorable attributes such as wide availability, fairly inexpensive, and it contains high quality proteins (16). The Coronary Artery Risk Development in Young Adults (CARDIA) study was a large community based observational study conducted in the U.S. with adults 18-30 years old, examined coronary risk factors. One of the findings of the study showed an inverse relationship between components of insulin resistance syndrome (including obesity) and dietary patterns characterized by dairy consumption (17). Dairy products are the focus of several studies due to both the micronutrient properties (calcium) as well as the macronutrient properties (protein, fat, and lactose). Previous research has shown that dairy products and their components are known to suppress short-term food intake and increase subjective satiety (66).

Milk proteins are more satiating than carbohydrates or fat, which can be explained by the interactions of the milk proteins on systems that control food intake (FI) and metabolism (67). Milk proteins contain all essential amino acids including high levels of branched chain amino acids (BCAAs) (37). In dairy, there are two main types of proteins; casein accounting for 80%

and whey protein(s) accounting for 20% (16). The two types of protein can be classified as a fast protein (whey), where amino acids are rapidly broken down and used to stimulate protein synthesis and slow protein (casein), where amino acid concentration is lower and broken down slower (68). Previous studies have examined the effects of protein in pre-load designs as well as within a meal. One study found that the addition of whey protein to a meal or as a pre-load was able to slow gastric emptying and also reduce post-prandial glycaemia in diet controlled type 2 diabetics (69). Nilsson et al. found that following a preload of 25g of carbohydrate with 18.2g of whey protein resulted in an insulin area under the curve (AUC) that was 50% higher than after milk or cheese (70). Milk proteins also increase cholecystokinin (CCK) concentrations, a known anorexigen (71), and can reduce plasma levels of ghrelin, the only hormone that stimulates food intake (72).

Currently, there is a large body of research investigating the role of macro- and micronutrients in glycemic regulation in the adult population. However, there is a lack of knowledge about glycemic regulation in children in response to the ingestion of certain foods. Therefore the aim of this study is to provide insight into the effect of snack products on glycemic control, satiety and energy intake in normal weight children.

Chapter 3
Rationale, Hypothesis, and Objectives

3.1 Rationale

Dairy products are available in a variety of food forms and have the potential to be a healthy snack option for children. This experiment will test the effect of a dairy product on blood glucose and insulin, satiety and food intake. It will compare dairy product with other non-dairy snack food product that is popular among children and has the same level of available carbohydrate.

3.2 Hypothesis

Dairy snack food provides better glycemic control, greater satiety, and reduces subsequent food intake at a test meal two hours later as compared to a commonly available non-dairy snack food.

3.3 Objective

To determine the effect of dairy products consumed two hours before a lunch meal compared with a popular non-dairy snack on blood glucose and insulin, satiety and energy intake in normal weight children.

Chapter 4

Methods

4.1 Experimental Design

Normal weight boys and girls, aged 9-14 years, participated in this randomized crossover study examining the effects of dairy and non-dairy snack on glycemic regulation, subjective appetite, and food intake. The study took place at the Canadian Center for Vaccinology (CCfV) at the IWK Health Centre in Halifax, NS. The participants came to the CCfV on two separate weekends on the same day at the same time. Participants were given a standardized breakfast (containing, milk, cereal, and orange juice) that was consumed at home two-hours prior to their arrival to the CCfV.

Once participants arrived, they were randomly assigned a dairy product (Liberte, Strawberry 0% M.F. Greek Yogurt, St. Hubert, QC) or a sandwich type cookie (Mini Oreo Mr. Christie's cookies, Kraft Canada, Don Mills, ON) treatment. Subjects were randomized to the treatments using randomization function in SAS software (SAS Institute Inc., Cary, NC). Both snack treatments contained the same amount of available carbohydrate (25g). Registered nurse took a baseline blood sample prior to the snack treatment and blood samples were taken at 30, 60, 90, and 120 min. Visual analogue scales (VAS) were used to measure subjective characteristics including appetite, physical comfort, and palatability of the treatments and test meal. The participants completed VAS measurements at 0, 30, 60, 90,120 minutes to assess motivation to eat (MTE) and physical comfort (PC). Following the 120 min blood draw, participants were given an *ad libtum* test meal of macaroni and cheese (Kraft Dinner *Easy Mac,* Kraft Canada, Don Mills, ON). Food intake was measured by converting the weight (g) of consumed test meal to its energy (kcal). The session day protocol is shown in the **Appendix 13**.

4.2 Participants

Sixteen participants were screened, twelve were eligible and interested in participating in the study, one participant decided not to return for the second session due to discomfort with the blood draw. Eligibility criteria included normal weight (5^{th}- $<85^{th}$ percentile based on CDC growth charts) and age 9 -14 years. Children were excluded from the study if they have food sensitivities or allergies, dietary restrictions, health, learning, emotional or behavioral problems,

if they were not born at term, or if they were receiving medication. The sample size of eleven was based on a previous study in young adults with a similar study design where a sample size of eight was able to detect significance of blood glucose concentration between different treatments (73). After eligibility was determined, each participant received a random code consisting of two letters following a number (i.e. JW-06). The code was used to identify the participant in all documents, files, and samples. Eleven normal weight participant ages 9-14 have completed the study.

Participants were recruited within the Halifax Regional Municipality. Recruitment occurred through the IWK CCfV participant database, IWK Facebook page, the MSVU nutrition studies database, HRM parent website, and through flyers (**Appendix 1)**. Parents of interested participants contacted the research group through the email or telephone. Once the contact was initiated, there was a two-stage recruitment process where participants were asked a series of questions through a telephone-screening interview to determine eligibility (**Appendix 2)**. When eligibility was confirmed based on the telephone screening, the guardian and participant came to MSVU for a screening session to further determine the participant's eligibility.

The in-person screening session involved gathering anthropometric data (weight and height) to determine the child's BMI based on the CDC growth charts. Body composition and weight was measured through a Bioelectrical Impedance Analysis (BIA), Tanita Body Composition Analyzer, (Tanita TBF-300A; Tanita Corporation of America, Inc., Arlington Heights, IL). Data was collected from the BIA for % fat mass and body weight (kg). The research assistant running the screening obtained age and height (using stadiometer) prior to using the BIA. Participants were told what the machine measures weight and fat mass by sending a tiny electrical impulse through the body that the participant cannot feel. The machine printed out the information and the research assistant recorded the participant ID and stored print out in the locked cabinet.

During the in-person screening, pubertal growth stage was measured through a Tanner Staging (74) (**Appendix 3)** as well as a puberty questionnaire (**Appendix 4)** and when relevant a menstrual cycle questionnaire (**Appendix 5).** The menstrual cycle questionnaire is used to determine the age of menarche as well as pubertal development. These questionnaires were presented to the parents first and then, if they agreed, to the participant. Parents chose to answer the questionnaire for the participant or the participant completed the form.

The participants completed a Dutch Eating Habit Questionnaire **(Appendix 6)** as part of the in-person screening. Van Strien and colleagues (75) developed the Dutch Eating Behaviour Questionnaire (DEBQ) in 1986. It consists of 20 questions, which are answered by "Yes", "Sometimes", and "Maybe". The DEBQ was intended to identify participants who display restrained, disinhibited, or emotional eating behaviours. The DEBQ was administerd during the on site screening session and was read to younger participants who have difficulty reading and interpreting the questionnaire. The results of this questionnaire can determine if the participant is a restrained, emotional, or external eater (75).

The participant were asked if they would eat the dairy and non-dairy treatments, and the test meal provided at lunch time, and the standardized breakfast that is provided prior to their weekend session at the IWK. The participants also completed a Physical Activity Questionnaire (**Appendix 7)**. This questionnaire was used in secondary data analysis to analyze hours per week spent doing physical activity and metabolic equivalents of the activities listed. Following the questionnaires, the participant and parents have reviewed and signed the Child Assent Form (**Appendix 8)** and the Parent Consent/ Study Information form, respectively (**Appendix 9).**

All procedures for this study were approved by the University Research Ethics Board at Mount Saint Vincent University (UREB File # 2011-025 & # 2012-063) and by IWK Research Ethics Board (IWK-REB Project#: 1008492). Copies of the parent and child assent forms were provided to the research nurses prior to the start of a session.

4.3 Dietary Treatments

Participants randomly received both the dairy and sandwich type cookie over the course of their participation in the study. The two treatments were used (a) Strawberry organic Greek yogurt (Liberté, St. Hubert, QC), (b) Mini Oreo Mr. Christie's cookies (Kraft Canada, Don Mills, ON) (**Appendix 12**). Both treatments contained 25 grams of available carbohydrate. The serving size for the yogurt was 199g with, 170.5 kcal, 0 grams fat, 17 grams protein, and 25 grams of available carbohydrate. The serving amount for the cookies was 37.5 grams with 175 kcal, 7.5 grams fat, 1.3 grams of protein, and 25 grams of available carbohydrate. The treatments were prepared and weighed each morning before the participants arrived at the IWK. The participants had an allotted time (8 minutes) to finish the treatment, which allowed for consumption of the food at a regular pace.

4.4 Experimental Protocol

4.4.2 Session Day

Participants came to the IWK for two sessions on the weekends and began the session at the same time. Prior to the session, participants consumed the standardized breakfast 2 hours prior to the scheduled start time. The standardized breakfast consisted of 250mL skim milk, 26 g of honey nut cheerios, and 236mL Tropicana orange juice (total kcal, 310). Once the participants have arrived at the CCfV, they were placed in a private patient room. Each participant was then asked a series of questions to confirm: they consumed the standardized breakfast, they had not eating anything 10-12 hours before the breakfast, and they have not eaten anything since the standardized breakfast**.**

Following the questionnaire, the research nurses applied an EMLA patch for 15-20 minutes (if the participant requests) to numb the skin. Before the baseline blood sample was taken, the participant filled out the baseline Visual Analogue Scale for subjective appetite (**Appendix 10).** Following the questionnaire, the baseline blood sample was taken. After baseline measurements were completed, the participant received the treatment randomly assigned for this session. Following the treatment, the participant was given another VAS form to assess palatability and sweetness of the treatment, and physical comfort (**Appendix 11).**

Nurses continued to take blood samples at 30, 60, 90, and 120 min and VAS was measured at the same time points. After the final blood draw (120min), the participants were offered a lunch (test meal) of macaroni and cheese (Kraft Dinner *Easy Mac,* Kraft Canada, Don Mills, ON). Prior to the first bowl of test meal, participants were told to turn off the TV and parents/siblings were instructed to leave the room. Each participant was served three separate bowls of macaroni and cheese and was given 10 minutes with each bowl. Once the first bowl of macaroni and cheese was given, participants were instructed to eat until they were comfortably full. Each bowl was made fresh and served to the participants within the same time frame (e.g. 5 minutes after it was cooked in the microwave). The participants were provided with a 500mL bottle of water at the start of the test meal was weighed to the nearest tenth of a gram prior to the meal and at the end of the meal to measure grams of water consumed. The mass of the consumed test meal (grams) was converted in energy (kcal) using the manufacturer's nutrition fact information. The session ended after the test meal and final the VAS is completed.

4.4.1 Blood Collection and Processing

Blood collections supplies such as alcohol swabs, tourniquets, and gauze were organized for blood collection. A nurse with a valid license to work in the Province of Nova Scotia completed the blood collection. The nurse wore non-latex gloves and used aseptic technique during phlebotomy. The gloves were worn during the handling and transport of specimens to minimize the possibility of direct contact. The nurse inserted the catheter and it was held in the arm for 2 hours, taking 3.5 ml samples of blood by attaching serum separating tubes (SST) tube at 0 min, 30 min, 60 min, 90 min, and 120 min, and P800 tubes at 0, 30, 60 and 120 min. For a visual representation of the time points and blood draws please refer to **Appendix 13.**

The venous blood collection system that was used is the shielded intravenous catheter (BD Insyte Autoguard™) connected with Luer-Access split septum device (BD Q-Syte™) and Luer-Lok™ access device (BD Vacutainer®)(**Appendix 13)**. The catheter and split septum device remained in the participants arm for the two hours and the access device was discarded after each blood draw. Following the blood draw, the split septum device was flushed with saline to eliminate syringe-induced blood reflux. This access system is optimal as it can reduce the risk of catheter-related bloodstream infection and provides a higher flow rate. The venous blood collection was collected in BD (Becton, Dickinson and Company, Mississauga, ON) serum separation (SST), 3.5 mL BD Vacutainer® Plus plastic serum tubes. Theses tubes are ideal for this purpose as they contain a clot activator and a serum gel separator.

SST tubes were labeled with the participant's ID, treatment's ID, date, and time point of blood collection beforehand. After blood collection, SST were inverted gently five times and stayed at room temperature for 30 minutes. The centrifuge was turned on prior to the start of the session to allow for the proper temperature to be reached (4°C). After the samples were spun, the serum was aliquoted into pre-labeled (participant ID, date of session, treatment, and time point) .5ml microtubes. The microtubes were then stored in appropriate boxes in a -20°C freezer until samples were ready to be analyzed (**Appendix 13)**.

4.5 Blood Glucose and Insulin

Samples were analyzed for blood glucose and insulin over 120 min for both treatments. Blood serum was analyzed for glucose using YSI 2300 STAT Plus Glucose Analyzer (YSI Life Sciences Yellow Springs, OH, USA), and for insulin using ELISA kit (ALPCO Diagnostics (Salem, NH, USA). The principal of YSI glucose analyzer system is based on YSI's enzyme sensor technology that produces hydrogen peroxide, which becomes oxidized producing

electrons, the electron flow is linearly proportional to the peroxide concentration and to the concentration of the substrate.

Serum insulin was measured through an enzyme-linked immunoabsorbent assay (ELISA). The assay was a sandwich type immunoassay that uses a microplate lined with monoclonal antibodies specific for insulin. The ELISA kit was supplied with the insulin standards and assayed human sera with known insulin concentrations with the insulin standards and assayed human sera with known insulin concentrations (Diabetes Controls). Once the samples were pipetted into separate wells the detection antibodies were added and the plate was placed on a microplate shaker for the first incubation. After the incubation period the plate was washed with a buffer solution and then a substrate was added to initiate the reaction. After the plate was incubated on the microplate shaker, a stop solution was added to end the reaction. In order to analyze concentration of insulin in the samples the plate was placed on a microplate reader where the optical density is measured by a spectrophotometer. The wells generated a color with the intensity that is directly proportional to the amount of insulin in the sample.

Following the analysis of blood glucose and insulin the concentrations were then used to detect difference by time and treatment. The results were also used to determine glucose to insulin ratios. Fasting glucose to insulin ratio (G:I) has previously been used in adults and adolescents as an inexpensive method for assessing insulin sensitivity (76, 77). In this study the participants are not fasted as they received a standardized breakfast two hours prior to their arrival in the lab, however it is still possible to compare baseline ratios between various groups of subjects and between the treatments. More recently G:I ratios have been calculated through using cumulative blood glucose to insulin ratios to determine if there are differences between pre-load treatments (78). For this experiment the cumulative blood glucose to insulin ratio was used to determine if there is a difference between the treatments.

4.6 Food Intake and Subjective Appetite

4.6.1 Food Intake

Food intake was measured through total energy consumed at lunch meal at time 120 min. The kcal consumed was measured by subtracting the initial weight of the bowl from the final weight of the bowl to determine energy consumed. The weight in grams consumed was then compared to the manufacturer nutrition label and the grams were converted into calories. Water

intake (WI) was measured through weighing the bottles prior to FI and then following the final bowl of macaroni and cheese.

4.6.2 Subjective Appetite

Visual analogue scales (VAS) are used to measure certain factors that can affect appetite sensations. These scales are important to short-term food intake research, as they are subjective measures that assess appetite. The scales are measured on a 100mm line that is anchored on both ends by opposing statements. Participants are then asked to make a mark on the line that corresponds with their feelings. The mark is then measured from the left to quantify their feelings. The visual analogue scales are often used in a pre-load paradigm following the test food as well as at specific time points until the 2nd meal is severed.

Reproducibility in VAS, when measured consecutively on separate days, shows high reproducibility in adults (79). In children, VAS measurements can be used to assess subjective appetite. Meal consumption reduces subjective appetite in 9-14 year old boys, which shows that children understand the concept of VAS (80). VAS demonstrates that children's subjective appetite scores show that meals decrease children's desire to eat and hunger (80, 81). A study with 9 to 10 year old normal weight children showed that when children did not consume breakfast they felt hungrier and that they could eat significantly more food before a lunch meal (82). However, the rating for prospective food consumption (i.e. how much they could eat) was not a reliable indicator of food intake for the lunch meal (82). Reliability of these scales is less variable when looking at repeat-reliability with group data averages versus individual subjects scores (83). Thus when using VAS, the average values over a specific time period has much better repeat-reliability than looking at scores of single time points (e.g. baseline) (83). The VAS measurements were used to determine differences between treatments, time, sex for all four measurements individually (DTE, Hunger, Fullness, and PFC) as well as using average appetite.

4.4.3.1Subjective Motivation to Eat

Subjective appetite of participants was measured through the use of the VAS. The MTE VAS consisted of four questions: 1) How strong is your desire to eat? (Desire to Eat, DTE) ('Very weak' to 'very strong'), 2) How hungry do you feel? ('Not hungry at all' to 'as hungry as I've ever felt'), 3) How full do you feel? ('Not full at all' to 'very full') and 4) How much food do you think you can eat? (Prospective food consumption, PFC) ('Nothing at all' to 'a large amount'). Participants will be asked to fill in the VAS for MTE at 0, 30, 60, 90, and 120 minutes.

Average Appetite was calculated using the following equation [average appetite (mm) = (desire to eat + hunger + (100 – fullness) + PFC) / 4] (80, 84, 85).

4.4.3.2 Subjective Palatability

The subjective palatability VAS assessed the participant's sensory perception of both the dairy and non-dairy treatment. Participants will be asked 'How pleasant have you found the beverage?' with the responses being, 'Not at all pleasant' and, 'Very pleasant.' This VAS was completed at 8 minutes following the consumption of the treatment.

4.4.3.3 Subjective Sweetness

The VAS measurement for subjective sweetness assessed how the participant found the dairy and non-dairy snack by asking 'How sweet have you found the beverage?' with the responses being, 'Not sweet at all' and, 'Extremely sweet.' This VAS was completed immediately following the consumption of the treatment.

4.4.3.4 Subjective Physical Comfort

The VAS measurement for Subjective physical comfort was measured by asking the question, 'How well do you feel?' with the responses being, 'Not well at all' and, 'Very well.' Participants have completed this VAS upon arrival (0 min), 8, 30, 60, 90, 120, and 150 minutes following the lunch meal.

4.4.3.5 Satiety Quotient

Satiety quotient (SQ) was used to determine the satiating effect of the treatments and has previously been used in other studies as a means to quantify satiety effect of a food based on subjective ratings of appetite and energy consumed (86, 87). The formula for satiety quotient is [(rating pre-eating episode –rating post-eating episode)/ intake of eating episode] (88). SQ can relate suppression of hunger to the energy consumed from a previous eating episode, and in the case of this study the dietary treatments (89). The SQ is a means of quantifying satiating power of a food that is useful when examining the effect of a pre-load or test meal on appetite over time as it allows researchers to test the satiating effect of a food at different time points (88, 90). A higher SQ indicates a greater satiating effect of the food or test meal, which varies over a period of time.

4.7 Data Analysis

Statistical Analysis Systems version 9.2 (SAS Institute Inc., Cary, NC) was used for all data analysis. Blood glucose and insulin concentrations were calculated for 0-120 minutes using

mean cumulative values and the total area under the curves (AUCs). Average appetite scores were calculated for 0-145 minutes using net area above the curve (AACs). If there is an influence of sex on the response to the treatment it was included in the model. To control for within subject variability all ANOVAs included session as a repeated measure. Three-way repeated measures ANOVA determined the effects of treatments, time, and the time-by-treatment interaction on blood glucose, insulin and average appetite scores for 0-120 minutes. To determine the effects of treatment at the specific time point's one-way repeated measure ANOVA was used. The effect of treatments on food intake and on blood glucose and insulin AUCs and average appetite AAC was determined by two-way repeated measures ANOVA. The mean differences among treatments are described through the Tukey-Kramer post-hoc test. All results are presented as mean ± standard error of the mean (SEM). The statistical significance is concluded with the P-value less than 0.05.

Chapter 5

Results

5.1 Participant Characteristics

Eleven children (6 girls and 5 boys) aged 9- to 14-years-old (11.9 ± 0.6 years), with a mean BMI percentile 54.7 ± 6.6 (BMI was between the 5th and 85th percentile range specific for age and gender), participated in the study. The mean body composition results are described by body weight (44.3 ± 4.3 kg), fat mass (7.7 ± 1.0 kg), fat free mass (FFM) (38.6 ± 3.9 kg), and total body water (38.6 ± 3.9 kg) **(Table 5.1).** Dutch eating behavior questionnaire results were broken into external eating (1.1 ± 0.04), restrained eating (2.1 ± 0.12), emotional eating (1.1 ± 0.04), and overall average DEBQ (1.4 ± 0.1) (**Table 5.1)**. The average Tanner stage was 1.7 for overall participants and 2.0 for boys (n = 5) and 1.5 for girls (n = 6) (**Table 5.1) (Table 5.2)**. The participants spent an average of 11 hours per week doing physical activity (**Table 5.1)** boys tended to have more physical activity per week than girls (11.5hrs vs. 10.7hrs) **(Table 5.1)**. The metabolic equivalents (METs) per week were 63.6 ml/kg/min and had a similar trend to physical activity when comparing boys and girls **(Table 5.1)**.

Table 5.1 Baseline Characteristics for All Test Participants

Subject Characteristic	All (N=11)	Boys (n= 5)	Girls (n=6)
Age (years)	11.9 ± 0.6	12.9 ± 0.8	11.0 ± 0.7
Weight (kg)	44.3 ± 4.3	52.5 ± 7.0	37.6 ± 3.9
Height (m)	1.5 ± 0.05	1.6 ± 0.08	1.4 ± 0.06
BMI (kg/m^2)	18. 5 ± 0.7	19.5 ± 1.2	17.6 ± 0.8
BMI %ile	54.7 ± 6.6	56.8 ± 7.5	53.0 ± 11.0
FM (kg)	7.7 ± 1.0	6.8 ± 1.2	8.5 ± 1.7
FFM (kg)	38.6 ± 3.9	45.6 ± 6.3	31.7 ± 2.0
TBW (kg)	28.3 ±2.9	33.4 ± 4.6	23.2 ± 1.5
DEBQ Average	1.4 ± 0.1	1.5 ± 0.2	1.3 ± 0.1
External Eating Score	1.1 ± 0.04	1.1 ± 0.03	1.1 ± 0.07
Restrained Eating Score	2.1 ± 0.12	1.9 ± 0.2	2.2 ± 0.2
Emotional Eating Score	1.1 ± 0.04	1.1 ± 0.03	1.1 ± 0.07
Tanner Stage	1.7 ± 0.33	2.0 ± 0.63	1.5 ± 0.34
PA Hours Per Week	11.04 ± 1.3	11.49 ± 0.62	10.67 ± 2.4
PA METs Per Week	63.55 ± 7.9	67.65 ± 8.6	60.14 ± 13.28

Data are means ± SEM, N = 11. Abbreviations: BW, body weight; BMI, body mass index; FM, fat mass; FFM, fat-free mass; TBW total body water; DEBQ, Dutch Eating Behaviour Questionnaire; PA, physical activity; METs, metabolic equivalents.

Table 5.2 Tanner Stages of All Participants

Tanner Stage	Number of Participants
Stage 1	7
Stage 2	1
Stage 3	2
Stage 4	1

5.2 The Composition of Dietary Treatments

The two treatments are (a) Strawberry organic Greek yogurt (Liberté, St. Hubert, QC), (b) Mini Oreo Mr. Christie's cookies (Kraft Canada, Don Mills, ON). Both treatments contained 25 grams of available carbohydrate. The serving size for the yogurt is 199g with, 170.5 kcal, 0 grams fat, 17 grams protein, and 25 grams of available carbohydrate. The serving amount for the cookies is 37.5 grams with 175 kcal, 7.5 grams fat, 1.3 grams of protein, and 25 grams of available carbohydrate (**Table 5.3**). There was no difference between the treatments for perceived sweetness ($P = 0.07$) or perceived pleasantness ($P = 0.4$) **(Table 5.3).**

Table 5.3 Nutritional Composition of Dietary Treatments

Nutrients[a]	Treatments	
	Greek yogurt (per 199 g)	**Cookies (per 37.5 g)**
Energy (kcal)	170.5	175.0
Fat (total) (g)	0	7.5
Protein (g)	17	1.3
Total Carbohydrate (g)	26.1	26.3
Sugars (g)	23.9	15.0
Fibre (g)	1.1	1.3
Sodium (mg)	62.5	212.5
Available carbohydrates (g)[b]	25.0	25.0
Sweetness (mm)	58 ± 8	75 ± 4
Treatment Pleasantness (mm)	72 ± 10	85 ± 8

[a] Nutrient content for each treatment as per Maxxam Analytics (Mississauga, ON) nutritional analysis results. [b]Available carbohydrates were calculated as a difference between total carbohydrates and dietary fibre.

5.3 Blood Glucose

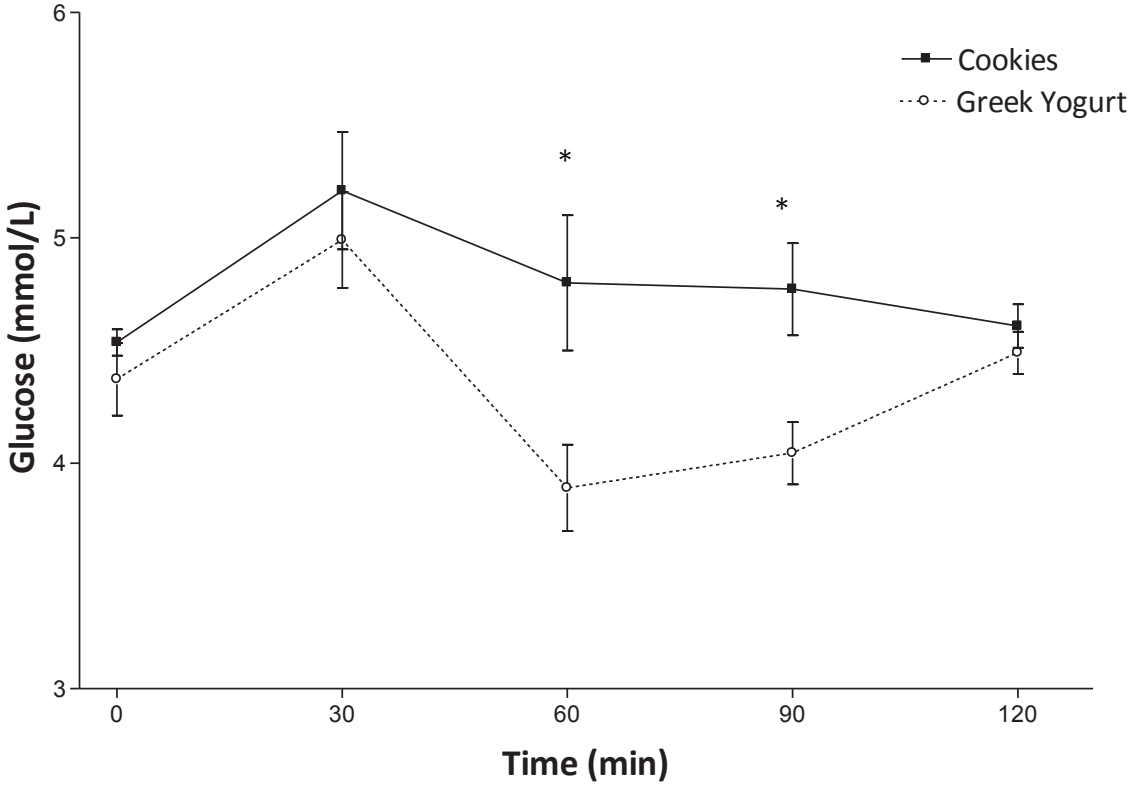
There was an effect of treatment (P=0.0013), time (P< 0.0001), and treatment x time interaction (P= 0.0382) on the mean cumulative blood glucose concentration and AUC over 120 min **(Table 5.4)**. Therefore, blood glucose was reduced overall following the dairy treatment when compared to the cookie treatment. The analysis of blood glucose at the individual time points showed that there was an effect of treatment, meaning blood glucose was reduced following the dairy treatment, at 60 min (P=0.0027) and 90 min (P=0.0102) **(Figure 5.1)**. There was no effect of sex on blood glucose concentrations (P=0.2) and there was a trend for the interaction between sex and treatment (P=0.06) for blood glucose values over 120 min. Blood glucose concentrations in girls were affected by time (P=0.004), treatment (P=0.01), and tended to have time-by-treatment interaction (P=0.06) **(Table 5.4)**. There was an effect of time (P<0.0001), and a trend towards a treatment effect (P=0.07) but no time-by-treatment interaction (P=0.14) for boy's blood glucose concentrations **(Table 5.4)**.

Table 5.4 Mean Value for Blood Glucose over 120 minutes

Treatment				
	Glucose (mmol/L)			
	All Participants (N=11)	**Boys (N= 5)**	**Girls (N=6)**	**tAUC (N=11)**
Cookies	$4.79 \pm .09^{1,2,3}$	4.51 ± 0.07^{1}	$5.01 \pm 0.15^{1,2}$	580.64 ± 22.22^{1}
Greek yogurt	$4.36 \pm .09^{1,2,3}$	4.32 ± 0.10^{1}	$4.39 \pm 0.14^{1,2}$	520.77 ± 15.45^{1}

Two-way ANOVA with a Tukey Kramer post-hoc test. Mean ± SEM. Superscripts indicate: [1]an effect of treatment; [2]an effect of time; [3]a treatment × time, P<0.05.

Figure 5.1 Blood Glucose Concentrations over 120 minutes



A one-way ANOVA with a Tukey Kramer post-hoc test. Mean ± SEM. Values with asterisk are significantly different (P<0.05)

5.4 Blood Insulin

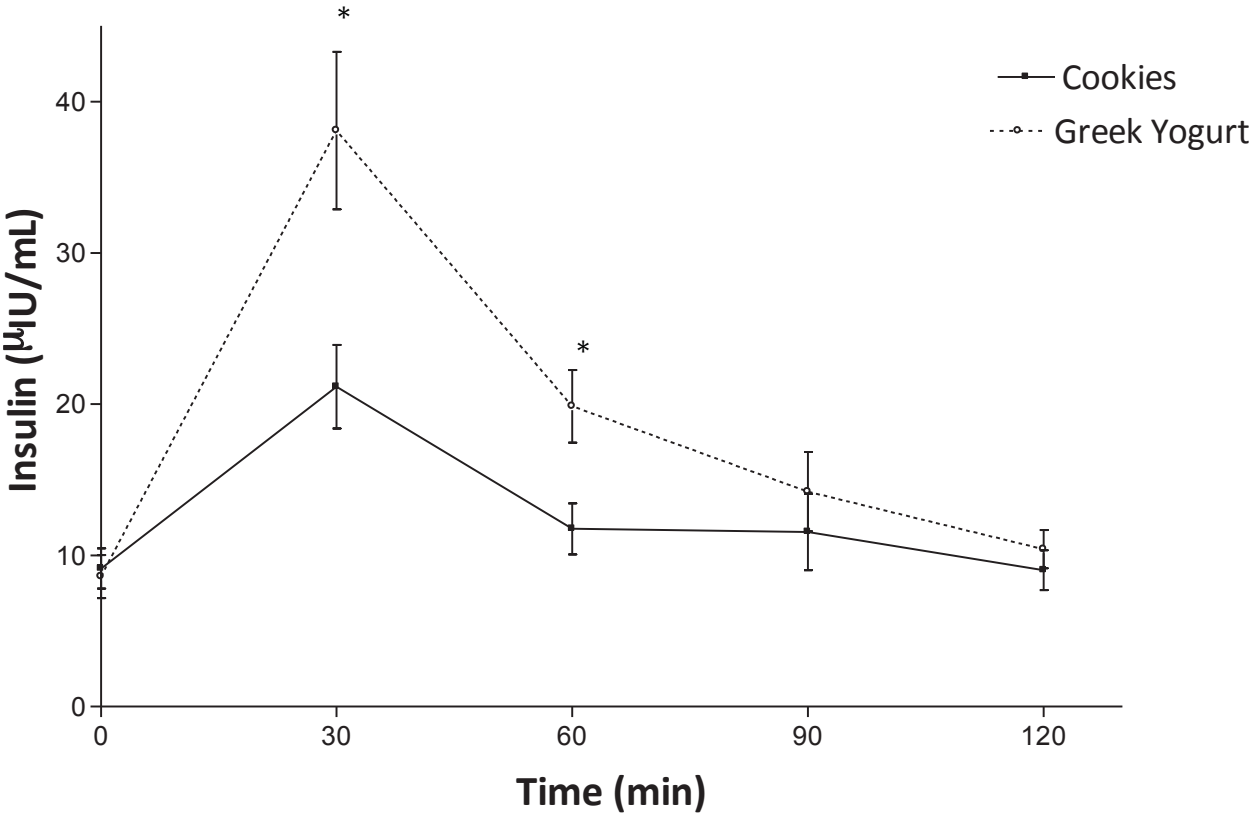
There was an effect of treatment ($P = 0.0001$), time ($P < 0.0001$), and treatment x time interaction ($P < 0.0001$) on the mean cumulative insulin concentration and AUC over 120 min (**Table 5.5**). Therefore insulin was greater overall following the dairy treatment compared to the cookie treatment. There was an effect of treatment on insulin at 30 min ($P = 0.0027$) and 60 min ($P = 0.0102$) indicating blood insulin was increased at these time points for dairy when compared to the cookie treatment **(Figure 5.2)**. There is an effect of sex on insulin values ($P = 0.0007$), meaning the girls tended to have higher mean insulin values overall, however there was not an interaction between sex and treatment ($P = 0.6$) for insulin values over 120 min. Girls insulin concentrations were affected by time ($P < 0.001$), treatment ($P = 0.002$), and a time-by-treatment interaction ($P = 0.0005$) was seen over 120 min (**Table 5.5)**. Insulin concentrations for boys were affected by time ($P < 0.0001$), treatment ($P = 0.009$), however no time-by-treatment interaction was observed ($P = 0.13$) **(Table 5.5)**.

Table 5.5 Mean Value for Insulin over 120 minutes

Treatment	Insulin (µIU/mL)			
	All Participant (N=11)	Boys	Girls	tAUC
Cookies	$12.52 \pm 1.06^{1,2,3}$	$9.17 \pm 1.53^{1,2}$	$15.3 \pm 1.37^{1,2,3}$	1606.93 ± 152.50^{1}
Greek yogurt	$18.23 \pm 1.92^{1,2,3}$	$13.64 \pm 2.15^{1,2}$	$22.06 \pm 2.89^{1,2,3}$	2320.02 ± 285.07^{1}

Two-way ANOVA with a Tukey Kramer post-hoc test. Mean ± SEM. Superscripts indicate: *[1]an effect of treatment; an effect of time; [3]a treatment × time, $P < 0.05$.*

Figure 5.2 Insulin Concentrations over 120 minutes



One-way ANOVA with a Tukey Kramer post-hoc test. Mean ± SEM. Values with asterisk are significantly different (P < 0.05).

5.5 Glucose to Insulin Ratios

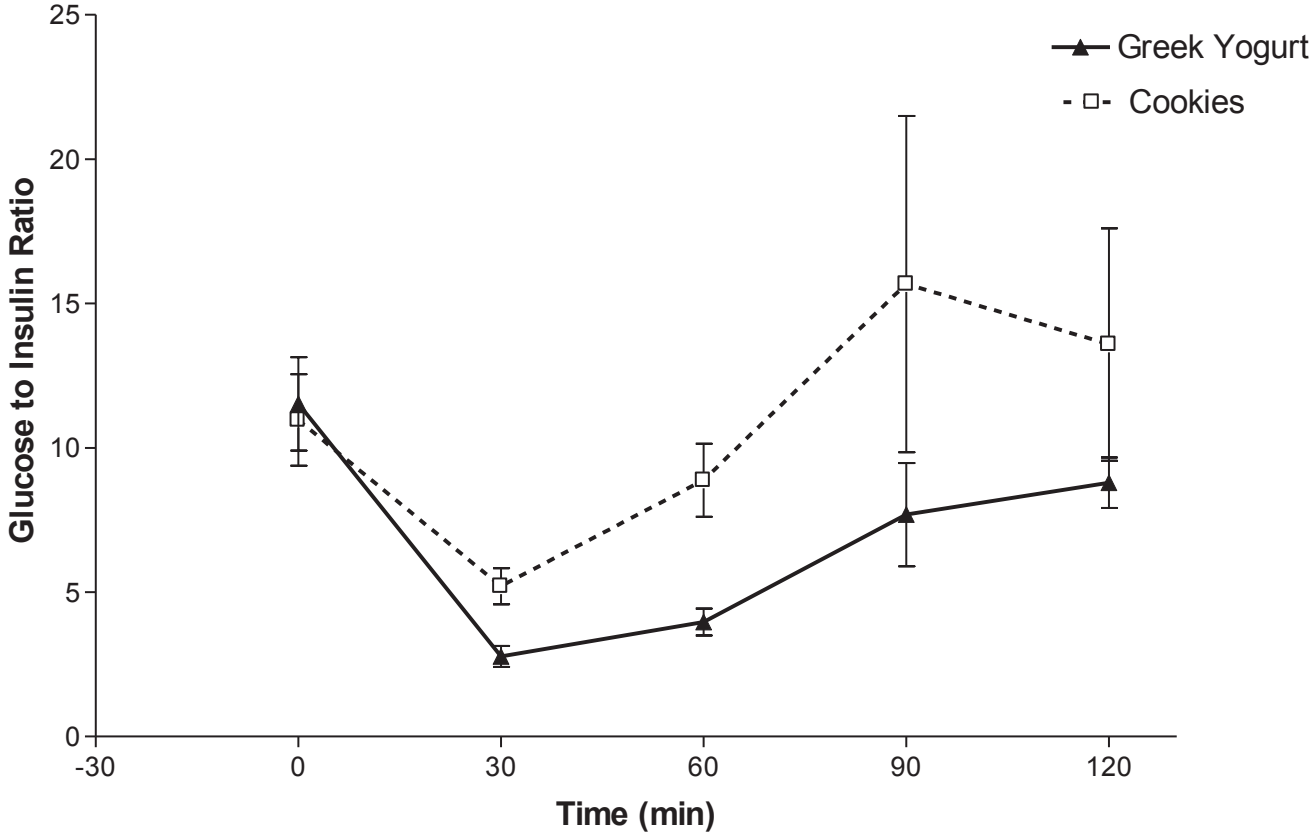
There was no effect of treatment (P = 0.4) or sex (P = 0.2) on baseline glucose to insulin ratios **(Table 5.6)**. The G:I ratios calculated from the mean cumulative glucose and insulin concentrations over 120 min was affected by time (P = 0.001) and treatment (P = 0.007) but there was no interaction between time and treatment (P = 0.04) **(Figure 5.3) (Table 5.6)**. G:I ratios calculated from the total areas under the curve (tAUC) were significantly different between the treatments (P = 0.008) **(Table 5.6)**.

Table 5.6 Glucose to Insulin ratios for Baseline and over 120 min

Variable	Baseline G:I	Cumulative G:I	tAUC G:I
Cookies	10.69 ± 1.56	10.86 ± 1.51[a,b,c]	6.60 ± 0.68[b]
Greek yogurt	12.48 ± 1.92	6.95 ± 0.67 [a,b,c]	4.69 ± 0.60[b]
Boys	13. 81 ± 1.74	12.24 ± 1.66[b,d]	7.11 ± 0.76[b,d]
Girls	9.73 ± 1.58	6.16 ± 0.44[b,d]	4.42 ± 0.37[b,d]

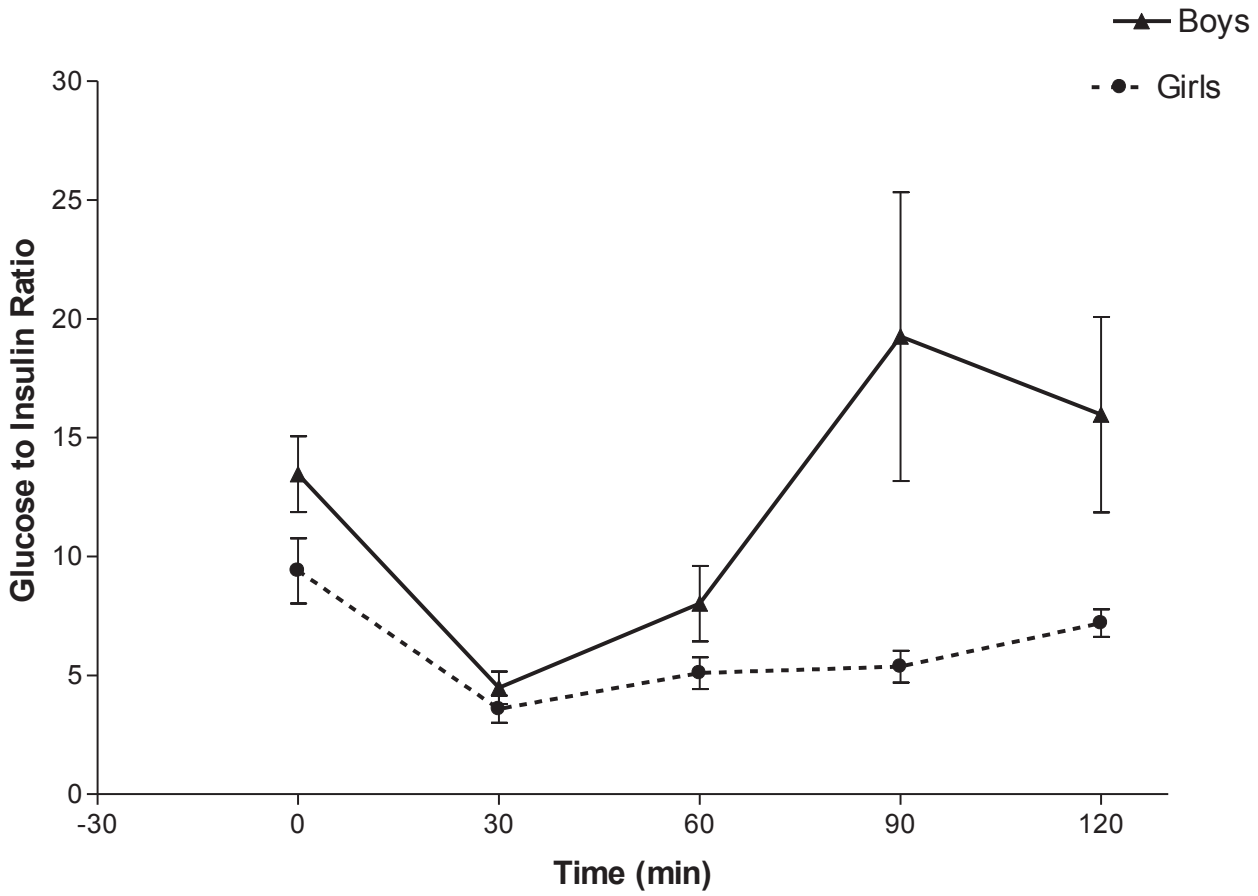
Two-way ANOVA with a Tukey Kramer post hoc test, Mean ± SEM. Superscripts indicate an effect of: [a]Time, [b]Treatment, [c] Time-by-treatment interaction, [d] Sex, P < 0.05.

Figure 5.3 G:I Ratios For Dairy and Non-Dairy Treatment over 120 Min



Means +SEM for all time points classified by treatment.

Figure 5.4 G:I Ratio Pooled Time and Treatments by Sex



Means +SEM for all time points and treatments classified by sex.

5.6 Ad libitum Food Intake and Subjective Appetite Scores

5.6.1 Food Intake

Food intake at the test meal was not affected by preload treatment (P = 0.91) **(Table 5.7)**. Water intake (WI) at the test meal was not affected by preload treatment (P = 0.37) **(Table 5.7)**.

5.6.2 Average Appetite

Average appetite (AA) scores were affected by time (P<0.0001) but there was no effect of treatment (P = 0.6) or sex (P = 0.5) **(Table 5.8)**. There were no significant interactions with subjective average appetite. AA scores increased overtime irrespective of treatment **(Figure 5.5a).** Changes from baseline AA scores were affected by time (P < 0.0001) but there was no effect of treatment (P = 0.7) or significant interactions **(Figure5.6a).** Total area under the curve (tAUC) for AA was not significant between treatments (P = 0.6) **(Table 5.9)**. The satiety quotient (SQ) for average appetite had an effect of time (P = 0.0001) and treatment (P = 0.014) but no time-by-treatment interaction (P = 0.5)(**Table 5.10 & Figure 5.7a**). The time interval 0-60 min for SQ average appetite was significantly different between treatments (P = 0.05) **(Figure 5.7a).**

5.6.3 Desire to Eat

Desire to eat (DTE) scores were affected by time (P < 0.0001) but there was no effect of treatment (P = 0.5) or sex (P = 0.5) **(Table 5.8)**. There were no significant interactions with DTE. DTE scores increased overtime irrespective of treatment **(Figure 5.5b).** Changes from baseline DTE scores were affected by time (P = 0.0003) but there was no effect of treatment (P=0.1) or significant interactions **(Figure5.6b).** Total area under the curve for DTE was not significant between treatments (P = 0.2) **(Table 5.9)**. Subjective desire to eat SQ was affect by time (P = 0.001), but not by treatment (P = 0.2), and there was no time-by-treatment interaction (P = 0.9) **(Table 5.10 & Figure 5.7b) (Figure 5.7b).**

5.6.4 Hunger

Hunger scores were affected by time (P = 0.0001) but there was no effect of treatment (P = 0.3) or sex (P = 0.4) **(Table 5.8)**. There were no significant interactions with subjective hunger. Hunger scores increased overtime irrespective of treatment **(Figure 5.5c).** Changes from baseline hunger scores were affected by time (P = 0.0003) but there was no effect of treatment (P = 0.2)

or significant interactions **(Figure5.6c).** Total area under the curve for hunger was not significant between treatments (P = 0.57) **(Table 5.9)**. The SQ for subjective hunger ratings had an effect of time (P = 0.0003) no effect of treatment (P = 0.9) and no time-by-treatment interaction (P = 0.95) **(Table 5.10 & Figure 5.7c).**

5.6.5 Fullness

Fullness scores were affected by time (P = 0.002) but there was no effect of treatment (P = 0.8) or sex (P = 0.7) **(Table 5.8)**. There were no significant interactions with subjective fullness. Fullness scores decreased overtime irrespective of treatment **(Figure 5.5d).** Changes from baseline fullness scores were affected by time (P = 0.003) but there was no effect of treatment (P = 0.3) and no significant interactions **(Figure5.6d).** Total area under the curve for fullness was not significantly different between treatments (P = 0.8) **(Table 5.9)**. Subjective fullness SQ was affected by time (P = 0.0007), but not affected by treatment (P = 0.1), and there was no time-by-treatment interaction (P = 0.8) **(Table 5.10 & Figure 5.7d).**

5.6.6 Prospective Food Consumption

Prospective food consumption (PFC) scores were affected by time (P = 0.0002) but there was no effect of treatment (P = 0.3) or sex (P = 0.3) **(Table 5.8)**. There were no significant interactions with subjective prospective food consumption. PFC scores increased overtime irrespective of treatment **(Figure 5.5e).** Changes from baseline PFC scores were affected by time (P=0.01) and treatment (P = 0.01) but there were no significant interactions **(Figure5.6e).** Total area under the curve for PFC was not significant between treatments (P = 0.4) **(Table 5.9)**. Subjective PFC SQ was affected by time (P<0.0001), treatment (P = 0.004), but there was not a time-by-treatment interaction (P = 0.8) **(Table 5.10 & Figure 5.7e).** Subjective prospective food consumption was significantly different at 0-120 min (P = 0.05) but not significantly different for 0-30min, 0-60 min, or 0-90 min **(Figure 5.7e).**

5.6.7 Physical Comfort

Physical comfort ratings were not affected by time (P = 0.70) or sex (P = 0.73) **(Figure 5.5f)** however; PC was affected by treatment (P = 0.01) **(Table 5.8)**. There were no significant interactions with subjective physical comfort. Changes in PC ratings from baseline were not affected by time (P = 0.93), treatment (P = 0.27), or sex (P = 0.7) **(Figure 5.6f).**

Table 5.7 Effects of Treatments on Food and Water Intake

	Greek yogurt	Cookies
FI[1] (kcal)	434 ± 53	439 ± 37
FI+Preload[2] (kcal)	605 ± 53	614 ± 37
Water Intake (g)	346 ± 95	308 ± 53

Mean ± SEM. Treatment effects were analyzed using the PROC MEANS with t-test for preload treatments as the main factors. Abbreviations: FI, food intake.

Table 5.8 Absolute Subjective Appetite Ratings

Appetite Rating		
	Greek yogurt	**Cookies**
Average Appetite (mm) [a]	52.75± 3.04	55.07 ± 3.75
Desire to Eat (mm) [a]	54.59 ± 3.27	52.51 ± 3.72
Hunger (mm) [a]	50.98 ± 3.26	53.82 ± 3.75
Fullness (mm) [a]	47.66 ± 3.25	48. 03 ± 3.92
Prospective Food Consumption (mm) [a]	54.38 ± 3.12	57.00 ± 3.73
Physical Comfort (mm) [b]	76.20 ± 2.98	85.11 ± 2.16

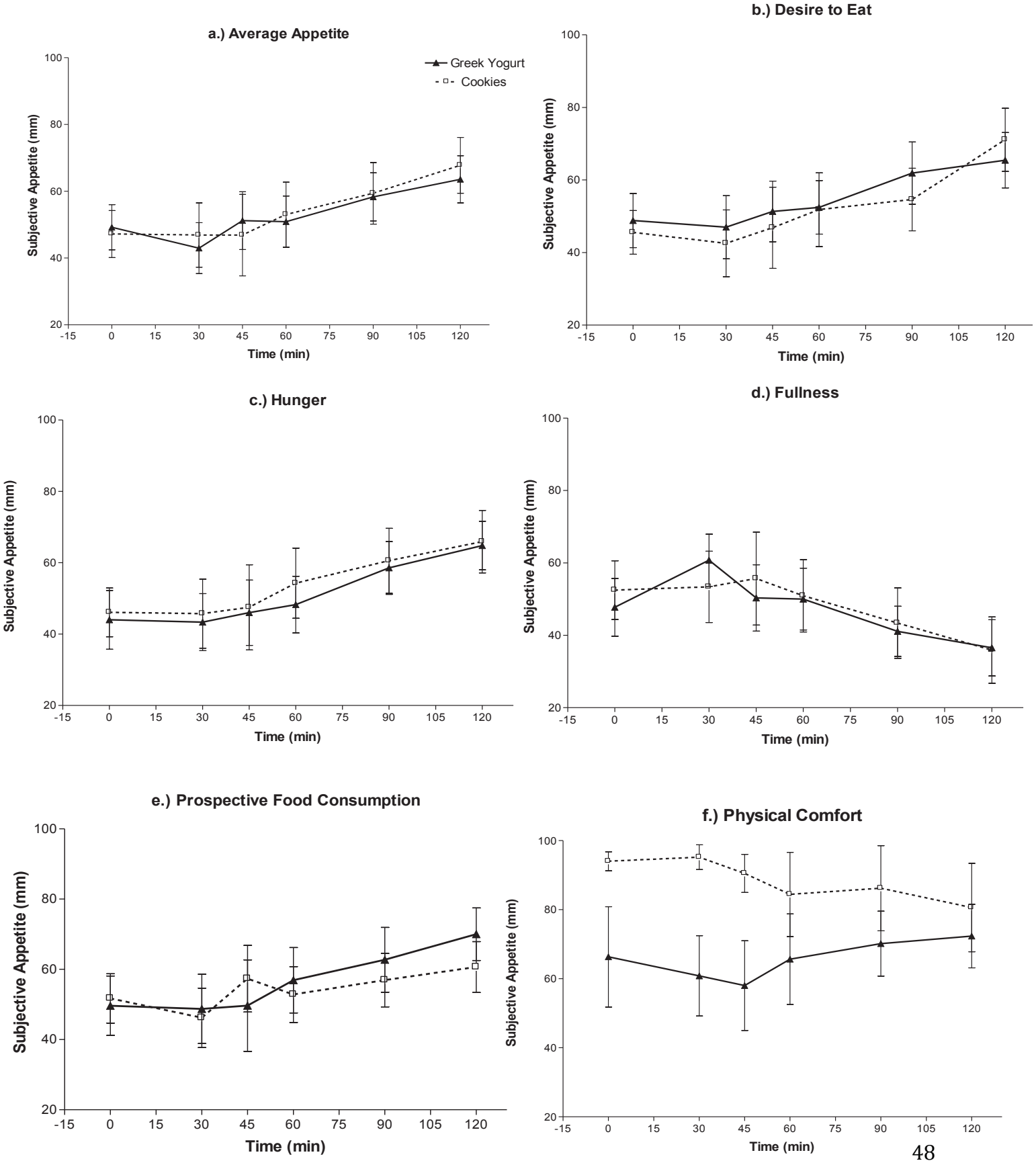
Two-way ANOVA with a Tukey Kramer post hoc test, Mean ± SEM. Superscripts indicate an effect of: [a]Time, [b]Treatment, P < 0.05.

Table 5.9 Total Area Under the Curve for Subjective Appetite Ratings

tAUC		
	Greek yogurt	**Cookies**
Average Appetite (mm x min)	6132.1 ± 822.2	5942.7 ± 917.1
Desire to Eat (mm x min)	4924.1 ± 656. 5	4284.6 ± 727.5
Hunger (mm x min)	4310.8 ± 557.3	4542.7 ± 666.1
Fullness (mm x min)	4830.0 ± 545.6	4592.3 ± 671.4
Prospective Food Consumption (mm x min)	4650.0 ± 557.3	4920 ± 305.5

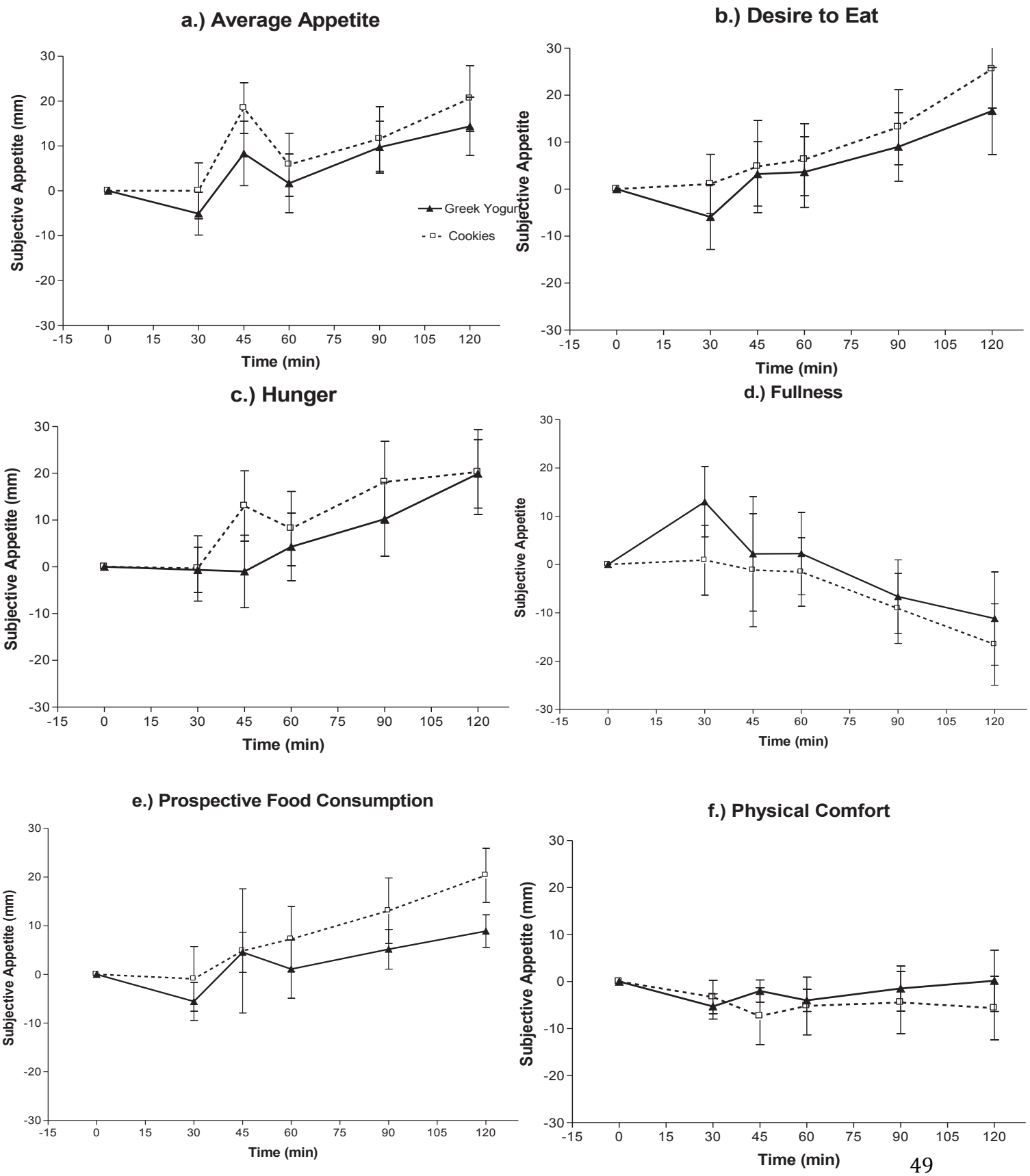
Treatment effects were analyzed using the PROC MEANS with t-test for preload treatments as the main factors.

Figure 5.5 Absolute Subjective Average Appetite After Treatments



Mean ± SEM.

Figure 5.6 Changes from Baseline Absolute Subjective Average Appetite After Treatments



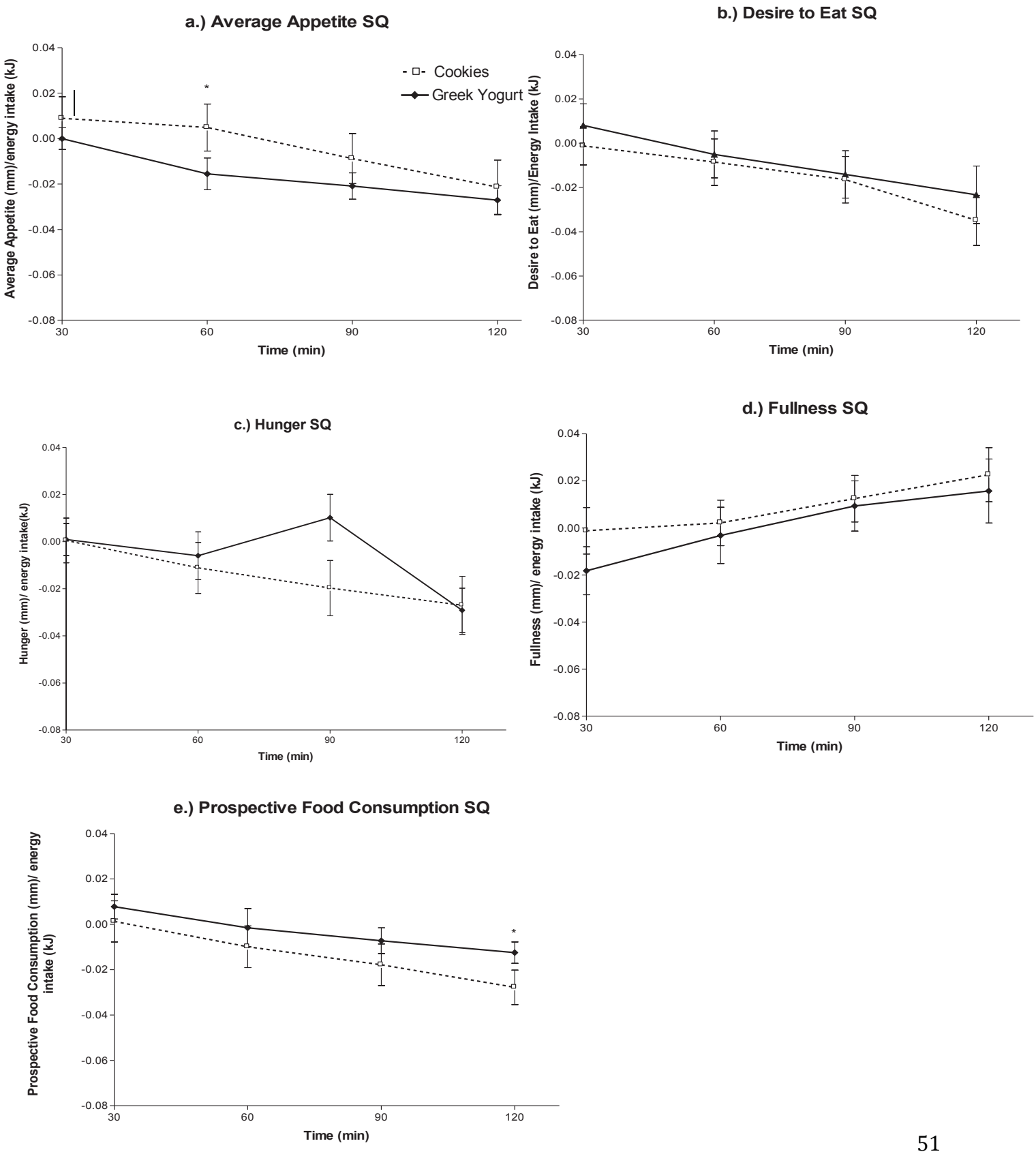
Mean ± SEM.

Table 5.10 Satiety Quotient for Dairy and Non Dairy Treatment

	Greek yogurt	Cookies
SQ Average Appetite[a,b]	-0.016 ± 0.003	-0.004 ± 0.005
SQ Desire to Eat[a]	-0.009 ± 0.005	-0.015 ± 0.005
SQ Hunger[a]	-0.014 ± 0.005	-0.014 ± 0.006
SQ Fullness[a]	0.001 ± 0.006	0.009 ± 0.005
SQ Prospective Food Consumption[a,b]	-0.003 ± 0.003	-0.014 ± 0.005

Two-way ANOVA with a Tukey Kramer post hoc test, Means + SEM. Superscripts indicate: [a] an effect of time; [b] an effect of treatment, P<0.05. Abbreviations: SQ, satiety quotient.

Figure 5.7 Subjective Average Appetite for Satiety Quotient Following Preload Treatments



Means +SEM. T-test for time points: asterisk indicates significantly different (P<0.05).

5.7 Relations among dependent measures

Mean BG scores for 0-120 minutes were not related to FI (r = +.03, P = 0.8). Total area under the curve for BG did not relate to FI (r = +.31, P = 0.16) **(Table 5.11).** BG at 120 min was not related to FI (r = -.16, P = 0.48) **(Table 5.11).** Insulin at 120 min was not related to FI (r = +.07, P = .75) **(Table 5.11).**

Subjective average appetite scores did not relate to FI (r = +.31, P = 0.16) **(Table 5.11).** Total area under the curve for hunger was not related to FI (r = +.34, P = 0.12) **(Table 5.11**). Total area under the curve for physical comfort ratings did not relate to FI (r = .29, P = 0.2). Perceived sweetness was not related to FI (r = +.06, P = 0.42) (**Table 5.11**). Perceived pleasantness was not related to FI (r = -.07, P = 0.78) **(Table 5.11)**.

Tanner stage was not related to FI (r = +.06, P = 0.37) **(Table 5.11).** Total area under the curve for insulin did not relate to Tanner Stage (r = -.28, P = 0.41) (**Table 5.12**). Mean insulin did not related to Tanner stage (r = -.29, P = .39) (**Table 5.12)**. Average baseline insulin for all participants on both treatment days did not relate to Tanner Stage (r = -.09, P = 0.79) (**Table 5.12).**

Mean BG for 0-120 min was not related to the mean average appetite scores for 0-120 min (r = -.10, P = 0.29) (**Table 5.13**). Mean subjective hunger ratings were negatively significantly related to mean subjective fullness ratings (r = -.71, P < 0.001) **(Table 5.14).**

BMI was not related to hours per week of PA (r = -.40, P = 0.22) or METs (r = -.33, p = 0.33) (Table 5.15). BMI percentile was negatively related to hours per week spent doing PA (r = -.82, P = 0.002) and METs per week (r = -.68, P = 0.02) **(Table 5.15).**

Table 5.11 Correlations with Food Intake

Variable	Food Intake
Mean BG 0-120min	r=0.02930
tAUC BG	r=0.31136
120min BG	r=-0.15770
120 min Insulin	r=0.07174
Average Appetite	r=0.31211
tAUC Hunger	r=0.34355
tAUC Physical Comfort	r=0.28522
Sweetness	r=0.18699
Pleasantness	r=-0.06589
Tanner Stage	r=-0.05666

*Pearson Correlation Coefficients,*Indicates P<0.05. Abbreviations, BG blood glucose; C, Cookies; GY, Greek Yogurt; tAUC, total area under the curve; PC, Physical Comfort; DEBQ, Dutch Eating Behavior Questionnaire.*

Table 5.12 Tanner Staging Correlations

Variable	Tanner Stage
tAUC Insulin	r = -0.27638
Mean Insulin	r =- 0.28883
Average Baseline Insulin	r = -0.09303

*Pearson Correlation Coefficients, *Indicates P<0.05. Abbreviations, tAUC, total area under the curve*

Table 5.13 Correlations with Average Appetite

Average Appetite Correlation	
	Average Appetite
BG for 0 -120 min	r = -0.10202

*Pearson Correlation Coefficients, *Indicates P<0.05. Abbreviations, BG, blood glucose.*

Table 5.14 Correlations with Hunger and Fullness

Subjective Appetite Rating	
	Subjective Fullness
Subjective Hunger	r = -0.7904*

*Pearson Correlation Coefficients, *Indicates P<0.05. Abbreviations, C, Cookies; GY, Greek yogurt*

Table 5.15 Physical Activity Correlation

Physical Activity Measurement		
	BMI	**BMI percentile**
PA Hrs./Wk.	r = -0.40314	r = -0.81880*
PA METs/Wk.	r = -0.32679	r = -0.68102*

*Pearson Correlation Coefficients, *Indicates P<0.01. Abbreviations, PA, physical activity; Hrs., hours; Wk., week; METs, metabolic equivalents*

Chapter 6

Discussion

The results of the study show that factors outside of the carbohydrate content of a snack can predetermine the glycemic response in normal weight children. In support of our hypothesis, the dairy snack provides better glycemic control compared to a commonly available non-dairy snack food.

The dairy snack resulted in a higher insulin level (at 30 and 60 min) (Figure 5.2) and lower blood glucose concentration (at 60 and 90 min) (Figure 5.3) compared to a non-dairy snack matched for available carbohydrate. When looking at the participants by sex, there is a trend towards an effect of treatment but it is most likely due to small sample size (boys, n= 5 and girls n=6) (Table 5.4). This finding was also in conjunction with the glucose to insulin (G:I) ratios results. G:I ratios have been used as a means to determine potency of insulin action (76, 77) and more recently used to detect differences in blood glucose and insulin concentrations overtime to compare different test foods (78). The results showed an effect of time as well as an effect of treatment when using cumulative and tAUC G:I ratios. Greek yogurt had a lower overall ratio compared to cookies indicating an increased efficacy of insulin action over 120 min for the dairy treatment (Figure 5.3).

A potential explanation for the difference in glycemic response between the two treatments may be the macronutrient composition, mainly the high protein and lactose content of Greek yogurt. Dietary proteins are an important macronutrient as they stimulates insulin secretion (91). Certain amino acids have a favorable effect on the pancreas as they stimulate beta cell function (92) and inhibit alpha cell function (93). More specifically dairy protein has previously been shown to have beneficial effects on glycemic response (70, 94). In a longitudinal study looking at young obese children the dairy rich group (instructed to consume >800 mg ca /d from low fat milk, cheese, and yogurt) showed significantly reduced HOMA-R and insulin levels at the12 month follow up compared to baseline values (95). The results of our study have shown similar results; the lower mean glucose seen in the dairy is the result of the insulin spike seen at 30 and 60 min. The mechanism behind the insulinotropic effect of dairy proteins remains unclear.

Dairy proteins may be unique as they contain varying degrees of rapidly digested protein (whey) and slowly digested protein (casein) (16). While the glucose reducing effect is well

known for whey protein, some studies showed that casein also reduces blood glucose. While the glucose reducing effect is well known for whey protein, some studies showed that casein also reduces blood glucose (96, 97). Greek yogurt used in this study is a commercially available product and the exact ratio of whey to casein is unknown, however typically Greek yogurt contains mostly casein (98). Therefore it is likely that casein is the main protein in the dairy product used in this study may play a role in the potential mechanism in the reduced glycemic response.

In the present study the insulin peaks for the dairy treatment occurred at 30 and 60 min, which was similar to the peak AA concentration seen for the whey, milk, and cheese meal (i.e. casein) in a study by Nilsson, (70) indicating that casein may have influenced this effect. Also, the insulin peaks seen in the present study at 30 min corresponds to the insulin peak seen after the cheese meal at 30 min in Nilsson's study (70). Casein has also been shown to be an influencing factor in glycemic response as it has an insulinotropic effect (99, 100). Recently, a randomized control trial has implicated casein as stimulant of insulin secretion in overweight adolescents (101).

Another potential mechanism could be a result of the type of carbohydrate found in the treatments. While the available carbohydrate was matched for treatments they contain varying degrees of specific carbohydrates. For example, lactose, when compared to sucrose or glucose, has been shown to have a lower glycemic response (71). The proximate analysis (Maxxam Analytics Inc.) of a similar product (President Choice Strawberry Yogurt, Loblaws Inc., Canada) showed that the lactose represents only 12% of carbohydrate content comprised mainly by scrose, glucose, and fructose. Therefore, such small content of lactose cannot explain the glycaemic response observed after the ingestion of yogurt. On the other hand, the high content of protein in yogurt treatment may explain the reduced glycemic effect observed after this treatment compared to the treatment with cookies that had 13 times lower protein content and similar content of glycemic carbohydrates (starch and sucrose). Although, the exact mechanism behind the elevated insulin concentration following the Greek yogurt cannot be explained in this study, a large body of research would support that it is likely due to the potent insulinotrophic effect of dairy proteins.

The variations in glycemic response and macronutrient composition between the dairy and non-dairy snack did not affect overall FI intake at 120min (Table 5.7). This finding was

similar to another study comparing dairy beverages to other commercially popular beverages, where differences in food intake were seen at 60 min but not at 120 min test meal (94). In respect to blood glucose and insulin results, there is a significant difference between treatments at 60 min however the effect is gone at 120 min. Another potential explanation could be that the energy content of each treatment was not large enough to create a difference in food intake. This was also seen in a study examining the effect of two yogurt snacks of varying protein content (matched for energy, 170 kcal) on an *ad libitum* meal. The results of that study showed no difference between the yogurt snack treatments on the *ad libitum* meal at 200 min (102).

When looking at subjective average appetite (AA) ratings there was an effect of time, showing that VAS are valid for assessing changes in appetite sensations in children, however there were no observed differences in subjective appetite between treatments (Figure 5.5a and 5.6a). The correlations with food intake showed there were no relationships with primary or secondary data outcomes (Table 5.11). Between the two treatments, appetite sensations of satiety are maintained for 45-60 min and following that time the sensations begin to decrease. The hunger ratings had a negative relationship with fullness ratings for both treatments, which highlights that the children and adolescents in the study could reliably indicate their subjective feelings of appetite (Table 5.12).

The AA scores were calculated to compare satiety quotients of the two treatments for participants. Satiety quotients have been used to quantify the satiating power of a food by relating subjective feelings of appetite to energy consumed (87). The results showed a lower satiety quotient over the 120 min for the cookie treatment indicating that participants found the cookies to be less satiating per kJ than the Greek yogurt per kJ. This finding is interesting as the treatments are nearly isocaloric (yogurt, 170.5 kcal vs. cookies, 175 kcal). However, there are key differences in the macronutrient composition (i.e. protein), which could be the reason for the difference of the satiating capacity seen between treatments as dairy proteins are also known to stimulate satiety (103). Also, when considering the satiating effects of pre-load over time, the satiety quotient for AA scores were significantly different from baseline at 60 min, meaning that the satiating effect of the treatments began to decrease at 60 min. This coincides with the significant differences seen in mean blood glucose values between the dairy and non-dairy treatment (60 min and 90 min).

Physical comfort ratings were not affected by time or sex but were affected by treatment (Table 5.8). Over the 120 min the participants rated higher feelings of comfort following the cookie treatment compared to the Greek yogurt. This could be explained by the difference in volume of the Greek yogurt (200 mL) compared to the cookies (125 mL). Participants may have been more full and less comfortable due to the difference in volume of the two treatments. Another potential explanation could be abdominal discomfort that can be seen with certain doses of lactose. Typically, traditional yogurt contains 7 – 9.7 g lactose per 200mL serving (104). The amount of lactose in the treatment is unknown however; typically Greek yogurt contains less lactose than regular yogurt due to the difference in processing. Abdominal discomfort has been shown to affect children who are maldigesters of lactose (insufficient lactase enzyme) when consuming 12 g lactose/ day (105). As none of the participants were lactose maldigesters, the amount of lactose in the product should have been well tolerated but may have contributed to participants feeling less comfortable with the dairy treatment. When looking at physical comfort there was an effect of session, indicating that participants were less comfortable on their first session than the second. This could be a result of participants being anxious at the first session possibly due to the venipuncture and anticipated level of discomfort. However, as this did not affect FI between treatments the physical comfort differences likely had little effect on the results. Also, physical comfort ratings were not related to food intake for either treatment (Table 5.11).

A potential confounding factor in the study was pubertal growth stage due to the transient insulin resistance state that occurs due to the increases in growth hormones, sex hormones, and insulin-like growth factor (22). Measuring pubertal growth is relevant as previous research has shown that girls who are peri-pubertal and post-pubertal respond to environmental cues differently which can affect short-term food intake (106). The self-administered Tanner stage questionnaire revealed that overall participants were pre-pubertal (Table 5.2). Also the results of the study show no effect of sex on baseline G:I ratios indicating uniformity of sample, which allowed for pooling of boys and girls (Figure 5.4).

The strength of the study is that we selected both treatments specifically because they are commercially available and typically consumed by children.

Chapter 7

Practical Applications

Dairy products and specifically Greek yogurt have favorable components that play a role in glycemic regulation by increased insulin secretion. This is especially important considering how dietary habits early in life can carry on to adulthood and interventions early on could prevent the health complications for children and adolescents. Dairy products are widely available and important sources of protein, vitamins, and minerals making dairy products a potential useful intervention in childhood obesity. The results of this study are important as dairy has shown to be a strong insulin inducer and could be potentially paired with high glycemic foods to help lower postprandial glycaemia.

Chapter 8

Conclusion

Dairy and non-dairy snack, matched for available carbohydrate, equally suppressed appetite and food intake at a second meal two hours later in 9-14 y children. The treatment with Greek yogurt significantly suppressed the glycaemia, due to increased insulin response compared to the treatment with cookies. Postprandial blood glucose level in children is predetermined by macronutrient composition of a snack rather than its available carbohydrate content only.

Chapter 9

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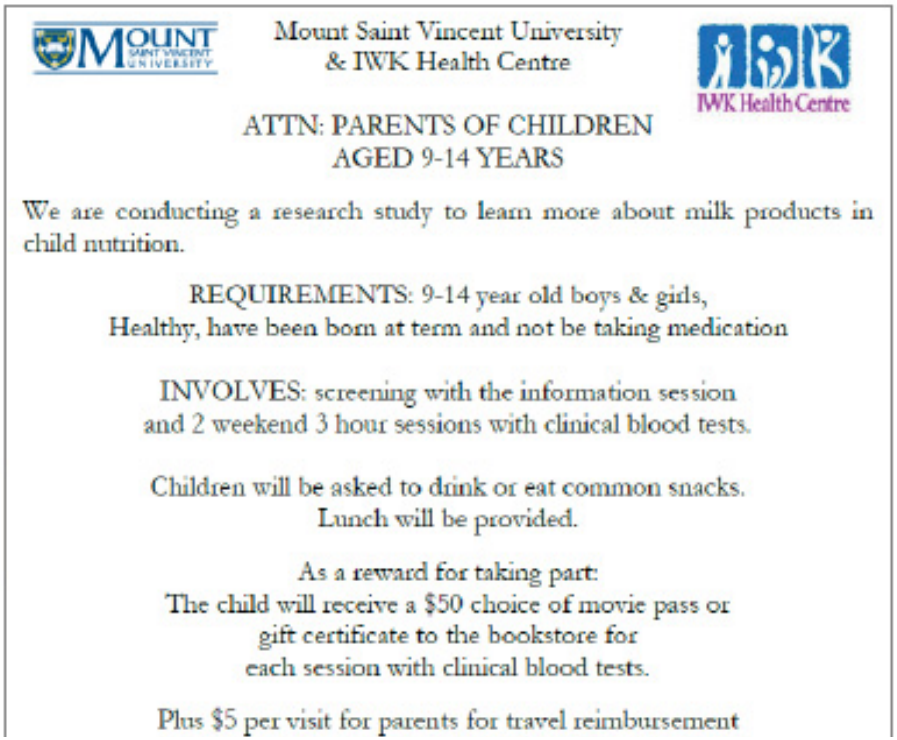
Chapter 10

Appendices

Appendix 1 Flyers for Recruitment



Appendix 2 Telephone Screening Sheet

The Effect of Milk Products on Glycaemic Regulation, Satiety and Food Intake in Children

Telephone Screening Questionnaire: part 1

Please print or circle the answer

Part: A / B

Name: _____ID assigned: _____

Age: _____ years

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

TO BE KEPT SEPARATELY

The Effect of Milk Products on Glycaemic Regulation, Satiety and Food Intake in Children

Telephone Screening Questionnaire: part 2

ID: _____

How many weeks gestational age? _____

What did your child weigh when (s)he was born? _____

Height: _____ cm Weight: _____ kg

Has your child lost or gained weight recently? Yes / No

Does your Child Usually have breakfast? Yes / No

Does your child like:

- Milk: Yes / No
- Yogurt (strawberry): Yes / No
- Cheese (mozzarella): Yes / No
- Cookies: Yes / No
- Potato Chips: Yes / No
- Orange Juice: Yes / No
- Honey Nut Cheerios: Yes / No

Is your child following a special diet? Yes / No

Does your child have any food allergies or food sensitivities? Yes / No

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

_____)

Medications?: Yes / No (If yes please explain: _____

_____)

Education: Grade: _____ Special Class? Yes / No

Has your child skipped or repeated a grade? Yes / No
(if yes which grade:_____)

Does your child have any learning difficulties/problems? Yes / No

(If yes please explain: _____)

Does your child have any behavioural or emotional problems? Yes / No
(If yes please explain:_____ _____)

Include in study? Yes / No

Appointment scheduled for: (date and time)

_____

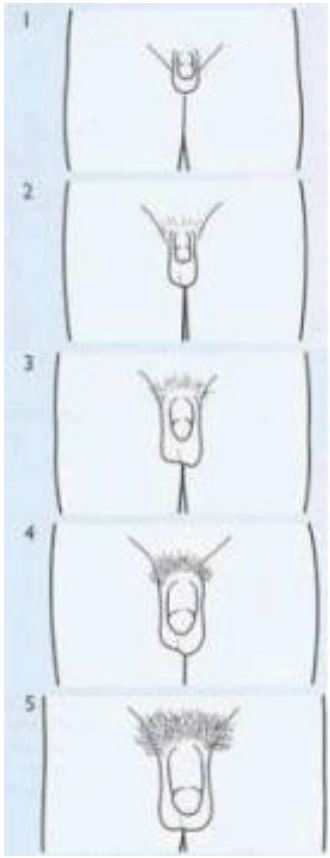
Investigator/Date screened:

_____

Appendix 3 Tanner Staging Questionnaire

Tanner Staging

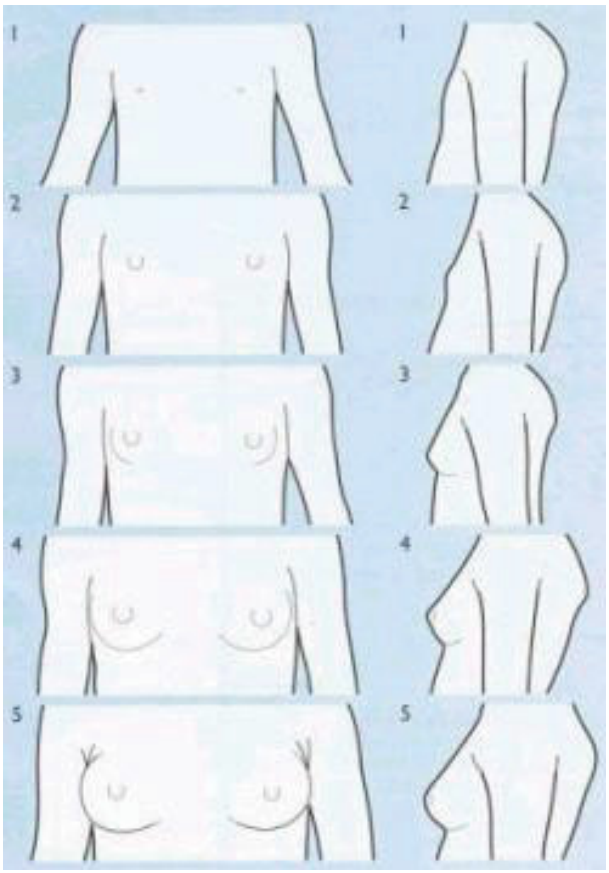
ID: _____ Date:_____



Tanner JM. Growth at adolescence; with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity: Springfeild, III, 1962

Tanner Staging

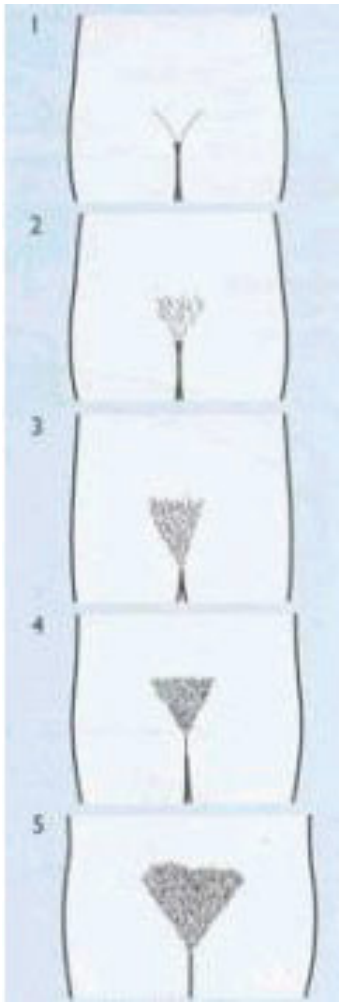
ID: _____ Date:_____



Tanner JM. Growth at adolescence; with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity: Springfeild, III, 1962.

Tanner Staging

ID: _____ Date:_____



Tanner JM. Growth at adolescence; with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity: Springfeild, III, 1962.

Appendix 4 Puberty Questionnaire

Puberty Questionnaire (Self-administered)

ID: _____ Date:_____

Would you say that your growth spurt (height):

1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

And regarding hair growth (under your arms, your pubic hair), would you say that:

1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

Have you noticed changes in your skin (e.g. acne)?

1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

FOR GIRLS:

Have your breasts started to develop?

1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

FOR BOYS:

Have you noticed that your voice has changed (lowered)?

1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

Have you started to have hair on your face?

1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

*NOTE: Girls with menarche start within a year of study visit = Tanner 4, girls with menarche start over one year of study visit = Tanner 5.

Appendix 5 Menstrual Cycle Questionnaire

Menstrual Cycle Questionnaire

ID: _____ Date:_____

1. When were you born? _____
2. Have you had your first period? _____

If you answered no, you are finished this questionnaire.

If you answered yes, please complete the following questions.

3. How old were you when you had your first period?

   I was _____ years old when I had my first period.

4. Do you remember the day/month of your first period? Yes/No

5. If you answered "yes", what was the date of your first period? _____

6. How long is your average menstrual cycle? (from the beginning of menstrual flow [menses] to the beginning of the next menstrual flow [menses])

   My average cycle length is _____ days.

7. Currently, for how many days do you typically experience menstrual flow each cycle?

   ____1 day____2 days ____3 days ____4 days ____5 days ___> 5+ days

8. In the past 3 months, estimate how many menstrual cycles you have had?

   I have had _____cycles in the past 3 months

9. In the past 6 months, estimate how many menstrual cycles you have had?

   I have had _____cycles in the past 6 months

10. In the past 9 months, estimate how many menstrual cycles you have had?

    I have had _____cycles in the past 9 months

11. In the past 12 months, estimate how many menstrual cycles you have had?

    I have had _____cycles in the past 12 months

12. How would you characterize your menstrual flow in the first two days of menses?

    Circle one: Heavy Moderate Light

13. Do you experience cramps during menses?

    Circle One: Always Sometimes Never

14. Do you typically experience any pain during the middle of your cycle?

    Circle one: Always Sometimes Never

15. Do you typically experience spotting or sporadic bleeding not associated with normal menstrual flow?

    Circle one: Always Sometimes Never

Appendix 6 Dutch Eating Behaviour Questionnaire

Dutch Eating Habits Questionnaire

1. Subject and test details

ID: _____

Age: _____

Gender: □ male □ female
Today's date: _____

2. Your weight, height, etc.

A. Current weight (kg): _____

B. Current height (cm)*:* _____

C. Has your body weight been constant over the past six months?

□ yes, my weight did not change much
□ no, I lost _____ kg
□ no, I gained _____ kg
□ no, sometimes I gained weight and sometimes I lost weight

D. Have you ever had an episode of eating an amount of food that others would regard as unusually large?

□ yes
□ no

Please do not mark below this line

BMI *(please take the age of the child into account)*: _____

DEBQ scale	Raw score	Number of items	Scale score	Classification
Emotional eating		7		
External eating		6		
Restrained eating		7		

Please turn over >>>>>

Instructions

Below you'll find 20 questions about eating. Please read each question carefully and tick the answer that suits you best. Only one answer is allowed. Don't skip any answer.

There are no incorrect answers; it's **your opinion** that counts.

1.	Do you feel like eating whenever you see or smell good food?	No	Sometimes	Yes
2.	If you feel depressed do you get a desire for food?	No	Sometimes	Yes
3.	If you feel lonely do you get a desire for food?	No	Sometimes	Yes
4.	Do you keep an eye on exactly what you eat?	No	Sometimes	Yes
5.	Does walking past a candy store make you feel like eating?	No	Sometimes	Yes
6.	Do you intentionally eat food that helps you lose weight?	No	Sometimes	Yes
7.	Does watching others eat make you feel like eating too?	No	Sometimes	Yes
8.	If you have eaten too much do you eat less than usual the next day?	No	Sometimes	Yes
9.	Does worrying make you feel like eating?	No	Sometimes	Yes
10.	Do you find it difficult to stay away from delicious food?	No	Sometimes	Yes
11.	Do you intentionally eat less to avoid gaining weight?	No	Sometimes	Yes
12.	If things go wrong do you get a desire for food?	No	Sometimes	Yes
13.	Do you feel like eating when you walk past a restaurant or fast food restaurant?	No	Sometimes	Yes
14.	Have you ever tried not to eat in between meals to lose weight?	No	Sometimes	Yes
15.	Do you have a desire to eat when you feel restless?	No	Sometimes	Yes
16.	Have you ever tried to avoid eating after your evening meal to lose weight?	No	Sometimes	Yes
17.	Do you have a desire for food when you are afraid?	No	Sometimes	Yes
18.	Do you ever think that food will be fattening or slimming when you eat?	No	Sometimes	Yes
19.	If you feel sorry do you feel like eating?	No	Sometimes	Yes
20.	If somebody prepares food do you get an appetite?	No	Sometimes	Yes

PLEASE CHECK TO BE SURE THAT YOU TICKED EVERY QUESTION

PAST YEAR PHYSICAL ACTIVITY

Check all the activities that you did at least ten times in the **PAST YEAR**. Include times spent in school physical education classes. Make sure you include all sport teams that you participated in during the last year.

____ Aerobics
____ Band/Drill Team
____ Baseball
____ Basketball
____ Bicycling
____ Bowling
____ Cheerleading
____ Dance Class
____ Football
____ Garden/Yard Work
____ Gymnastics
____ Hiking
____ Ice Skating
____ Roller Skating
____ Running for Exercise
____ Skateboarding
____ Snow Skiing
____ Soccer
____ Softball
____ Street Hockey
____ Swimming (Laps)
____ Tennis
____ Volleyball
____ Water Skiing
____ Weight Training
____ Wrestling (Competitive)
____ Others

_____
_____
_____

List each activity that you checked above in the "Activity" box below, check the months you did each activity and then estimate the amount of time spent in each activity.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Months Per Year	Days Per Week	Minutes Per Day

IWK Health Centre
5850/5980 University Avenue
PO Box 9700
Halifax, NS B3K 6R8
Canada
Tel: 902.470.8888
www.iwk.nshealth.ca

Appendix 8 Child Assent

Appendix 1c

The Effect of Milk Products on Glycaemic Regulation, Satiety and Food Intake in Children

Children's Assent Form

We are doing a research study to find out how good some snacks and drinks are for children's health. You might want to take part. It is your choice. No one will be mad at you if you do not want to take part.

Here's what you will do if you decide to be in the study:
Come to Mount Saint Vincent University with your parent on one day and we measure your body weight, height and ask questions about what do you like and don't like to eat, and some questions about how you grow. On two other days you will come to the IWK Health Centre for a snack session. While you are there, we will take some blood samples to measure your blood sugar levels before and after your snack. The nurse will put a tiny soft plastic tube in your arm so she can take all the samples without poking you more than once. If you want, she can use numbing cream on the spot before she puts the needle in, so it doesn't hurt.

There will be other children like you at each session too. We will ask you and your parent to answer some questions about your health and how you are growing up. You do not have to answer any questions if you are feeling shy. We will see how tall you are and how much you weigh. We will measure to see how much body fat you have. It will not hurt. You will not have to miss school because the research will happen on days off.

If you start taking part and decide you want to stop, that is OK. Just tell your parent or the people at the session and you can go home.

To say thank you, we will give you a gift certificate for each session you attend, and your parent will get some money for parking.

"I was present when _____read this form and gave his/her verbal assent."

_____ Signature

Name of the person who obtained assent:

_____

Appendix 9 Parental Consent Form

Appendix 1b
The Effect of Milk Products on Glycaemic Regulation, Satiety and Food Intake in Children

Study Information Sheet and Parent's Authorization Form

Investigators:

Dr. Younes Anini, Investigator
Reproductive Endocrinology and Neuroendocrinology Lab
Department of Obstetrics and Gynaecology
Dalhousie University, IWK Health Centre
Phone: [redacted]
Email: [redacted]

Dr. Bohdan Luhovyy, Investigator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: ([redacted]
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Dr. Nick Bellissimo, Investigator
School of Nutrition, Ryerson University
Phone: ([redacted]
Email: [redacted]

Dr. G. Harvey Anderson, Principal Investigator
Department of Nutritional Sciences, University of Toronto
Phone: [redacted]
Email: [redacted]

Dr. Jill Hamilton, Investigator and Pediatrician
Department of Paediatrics, University of Toronto
Phone: [redacted]
Email: [redacted]

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Department of Applied Human Nutrition, Mount Saint Vincent University
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Ms. Mary McCormick, P.Dt. M.Sc. Student
Department of Applied Human Nutrition, Mount Saint Vincent University
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Ms. Tove Armstrong, Research Assistant and Study Coordinator

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Mr. Brandon Gheller, B.Sc. Research assistant
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: [redacted]
Email**:** [redacted]

INTRODUCTION

Your child is invited to take part in the research study named above. This form provides information about the study. Before you decide if you want your child to participate, it is important that you understand the purpose of the study, the risks and benefits and what you and your child will be asked to do. We will provide you with information before asking for your authorization to participate. We will keep you informed of any new information that might affect your willingness to continue participating. A member of the research team will be available to answer any questions you have. You may decide not to have your child participate or you may withdraw your child from the study at any time. Your child does not have to take part; it is entirely voluntary (your choice). Your decision will not affect the care you or your family members receive from the IWK Health Centre in any way.

Why are the researchers doing the study?
Canada's Food Guide recommends 3-4 servings of dairy products a day for children 9-18 years old. Research shows that they eat fewer than 2 servings. In the last 30 years, fewer children drink milk and more children are overweight. More children are being diagnosed with type-2 diabetes. Children have replaced dairy product snacks with sugar-sweetened beverages and foods high in calories and poor in nutrients. Therefore the purpose of this study is to determine how dairy product snacks affect the amount of food eaten at the next meal, calories eaten and blood sugar levels in children age 9 to 14 years. We hope to use this information to help prevent obesity and diabetes in children in the future.

This experiment is being conducted through the Departments of Applied Human Nutrition at Mount Saint Vincent University and Nutritional Sciences at the University of Toronto by Dr. Bohdan Luhovyy, Dr. Nick Bellissimo and Dr. G. Harvey Anderson. Your son/daughter will be required to attend two experimental sessions conducted over a 2-week period and one screening session to measure blood sugar and insulin for a total of 3 visits (1 screening session + 2 experimental sessions) to the Mount Saint Vincent University campus for screening and to IWK Health Centre for clinical tests. This study is a randomized clinical trial which means that your child will be asked to consume a randomly selected food product (yogurt or cookie). For example your child will be asked to consume yogurt on the first session and cookies on the

second session, or backward cookies on the first session and yogurt on the second session. Then we collect a small amount of blood (35 ml per one session which is about two table and one tea spoons) to measure blood sugar and insulin. The maximum duration of the session will be 3.5 hours. The test day starting from the breakfast at home until the end of the session will last no more than 5.5 hours.

This study will not cost you anything. Your child will receive $50 gift card for each session and you will be compensated for the transportation ($5 per session day).

We anticipate having about 40 kids enrolled in this study which is the part of large research project that is financially supported by Dairy Farmers of Canada. Another study of this project is conducted in the University of Toronto. There are no conflict of interests between instigators, participants and the sponsor.

PROCEDURE:

Screening:

If you agree and your child wishes to participate, we will measure his/her weight, height, and body fat. None of these measurements will hurt.

Your child will be asked to complete two questionnaires that will help us to assess your child's physical activity and eating habits. Our Research Assistants will help your child if necessary and answer all questions that your child may have.

- Menstrual Cycle Questionnaire:

Girls will be asked to complete a questionnaire about their menstrual cycle. This information is collected because studies have shown that energy intake and appetite change across the menstrual cycle.

- Tanner Staging:

To assess the effect of pubertal stage on food intake in children, a questionnaire relating to puberty and 3 cartoon images will be administered to the children in lieu of an examination. Your child will be asked to circle the number on the side of the picture that best represents him/her. Tanner stages are scales that assess physical development in children and adolescents, based on external primary and secondary sex characteristics, such as the size of the breasts, genitalia, and development of pubic hair. The way in which appetite is regulated is related to where children are in their pubertal development. If for any reason your child is not willing to participate, he/she has the option of ask you to answer the questionnaire and select the pictograms for him/her. Your child may decline the pubertal staging if he/she wishes. Parents are welcome to discuss the reasons for

including Tanner stages as part of the study or any comment or concerns with Dr. Jill Hamilton at [redacted]

- Body Composition Assessment:

The painless method of bioelectrical impedance analysis will be used to estimate the amount of muscle and fat tissue in your child's body.

Bioelectrical Impedance Analysis: Bioelectrical impedance analysis (BIA), a recently developed technique for measuring body fat content in both adults and children, is simple and painless and is an effective method for measuring body fat in children. BIA is based on measurement of electrical resistance in the body to a tiny current (that the child cannot feel). The principle of BIA lies in that muscle mass in the body is a better conductor of electricity than fat which contains lesser amounts of water and electrolytes.

- Blood Sugar and Insulin Assessment:

Your child will be asked to go to the IWK Health Centre for two individual morning sessions. These sessions will be held on weekends, over two weeks. Please note that children will be brought to the laboratory and returned home by parents only.

On each of the two test days, your child will have a standardized breakfast of cereal, milk and juice at home, either at 7:00 am or 7:30 am (the time must be consistent for each session day). The children will arrive at the IWK Health Centre, either at 9:00 am or 9:30 am (but consistent throughout for each session day).

Your child will fast for 12 hours before breakfast and after breakfast until he/she arrival, except for water, which will be allowed up to one hour before their arrival.
At each experimental session, blood will be sampled and used to measure blood sugar and insulin. Five blood samples will be taken during each experimental session. The total volume of blood collected at one session will be no more than 35 ml (two table and one tea spoons) and the total volume of blood collected within two weeks will be 70 ml at the most. To obtain blood samples, a registered nurse will insert a catheter (a needle attached to a plastic tube) into a vein in your child's arm. Your child may choose to use a cream to numb a skin (Emla or topical anaesthetic cream). The catheter will remain in his/her arm and be used to sample blood in small amounts for 2 hours. During these two hours, your child will be asked to avoid any activities such as active gaming. After the nurse collects the first sample at baseline (0 minutes), your child will be given one of the following product at each session: yogurt, or cookies. Your child will be asked to consume the whole portion of the product that will be provided. After then, we will collect blood samples at 30, 60, 90 and 120 minutes after baseline. The second attempt to insert catheter will be made only if your child is comfortable doing this**.** Blood samples will be stored in the laboratory until analyzed and then safely discarded.

Your child will be fully supervised during the study sessions. He/she will be involved in age appropriate entertainment (as distraction) e.g.: reading, puzzles, cards, before lunch. There will be other children there participating in the study. After the session, your child will be provided

with a lunch to rehydrate and replenish energy. You can pick your child up in 2 hours or you have an option to stay with your child is you wish.

CONFIDENTIALITY:

Records relating to your child will be kept confidential in a locked cabinet in the Department of Applied Human Nutrition and no disclosure of personal information of the children or parents will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data from children who have completed the study will be entered into Microsoft Excel files, available only to investigators. Each child will have a file, also only available for investigators. If your child will withdraw from the study, all her/his data will be removed and all hard copies will be destroyed. Please note that choosing to withdraw will not affect care at IWK. All forms and printouts will be stored in the individual files and clearly labeled. All documents will be kept for a minimum of five years following publication of the study and then securely destroyed. No disclosure of personal information of children or parents will take place except where require by law, for example concerns of suspected child abuse.

RISKS:

There is very little risk related to this study. There is the possibility of a small amount of bruising, pain and the possibility of infection associated with blood collection.
The provided snacks are commercially available and safe for human consumption. Children may feel dizzy following the overnight fast, but this is rare. If this happens, they will likely feel fine once they consume the breakfast meal provided. There is a possibility of other unexpected risks.

BENEFITS:

As the causes of obesity remains undefined, the potential benefits from this study will be a better understanding of the regulation food intake in children and might contribute to the prevention of obesity in children. Each child will receive a copy of Canada's Food Guide along with a copy of "My Food Guide" personalized for each child.

QUESTIONS AND FURTHER INFORMATION:

Participation is completely voluntary and failure to participate will not have any consequences. Also, you and your child have the option to stop participating, skip any step/question or withdraw from the study at any time.

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Dr. Bohdan Luhovyy ([redacted] You can also contact our Study Coordinator Ms. Tove Armstrong at ([redacted] and leave a message. We will call you back shortly.

We may want to contact you in future to provide information about our other projects you or your child may be interested in and invite your child to participate in these projects.

RESEARCH RIGHTS:

Your signature on the form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigator(s), sponsors, or involved institution(s) from their legal and professional responsibilities. If your child becomes ill or injured as a direct result of participating in this study, necessary medical treatment will be available at no cost to you. You are free to withdraw from the study at any time without jeopardizing the health care you are entitled to receive.

If you have any questions at any time during or after the study about research in general you may contact the Research Office of the IWK Health Centre at ([redacted], Monday to Friday between 8:00a.m. and 4:00p.m.

I understand that for purposes of the research project, if my child or I choose to withdraw from the study at any time, we may do so without any problems.
Upon completion of each study session, my child will receive a $50 gift certificate to the theatre or bookstore. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter's identity.

RESEARCH RESULTS:

If you wish, a summary of the study results can be provided. They will be available around one year after the end of the study.

The Effect of Milk Products on Glycaemic Regulation, Satiety and Food Intake in Children

Participant ID: _____

PARENT AUTHORIZATION:

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks. I understand that my child and I have the right to withdraw from the study at any time without affecting the care my family and I will receive in any way. I have received a copy of the Information and Authorization Form for future reference. I freely agree to participate in this research study.

Would you like to receive a summary of the results when they are available? ___ Yes,___ No.

Would you like to be contacted for future research? Yes___, No____.

Name of Participant: (Print) _____

Name of Parent: (Print) _____ Parent Signature: _____

Date: _____ Time: _____ Participant ID: _____

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT

I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Name: (Print) _____

Signature: _____ Position: _____

Date: _____ Time: _____

Appendix 10 Visual Analogue Scales, Motivation to Eat

Visual Analogue Scale
Motivation to Eat

DATE: _____ Session _____

Treatment ID ____

ID: _____ Time point ____ min

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 WEAK _____ Very STRONG

2. How hungry do you feel?

NOT Hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

NOT Full at all _____ VERY Full

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

NOTHING at all _____ A LARGE amount

Appendix 11 Treatment Palatability

Visual Analogue Scale
Pleasantness and Sweetness

DATE: _____ Session _____
Treatment ID ____
ID: _____ Time point ____ min

This question relates to the palatability of the food you just consumed. Please rate the pleasantness of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

How pleasant have you found the drink/food?

NOT at all pleasant _____ Very pleasant

How pleasant have you found the drink/food?

NOT at all pleasant _____ Very pleasant

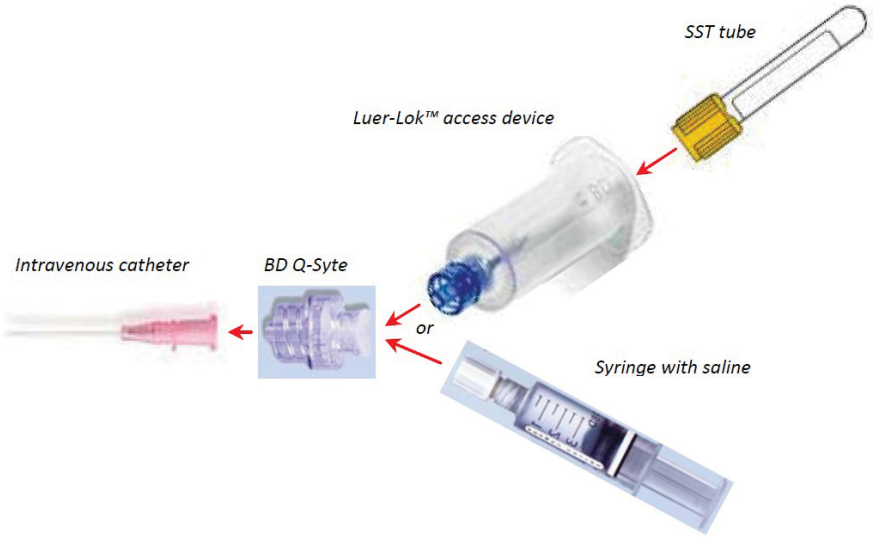
Appendix 12 Proximate Nutrient Analysis for Dietary Treatments

Dietary Treatments for the study: Energy and Macronutrient Content:

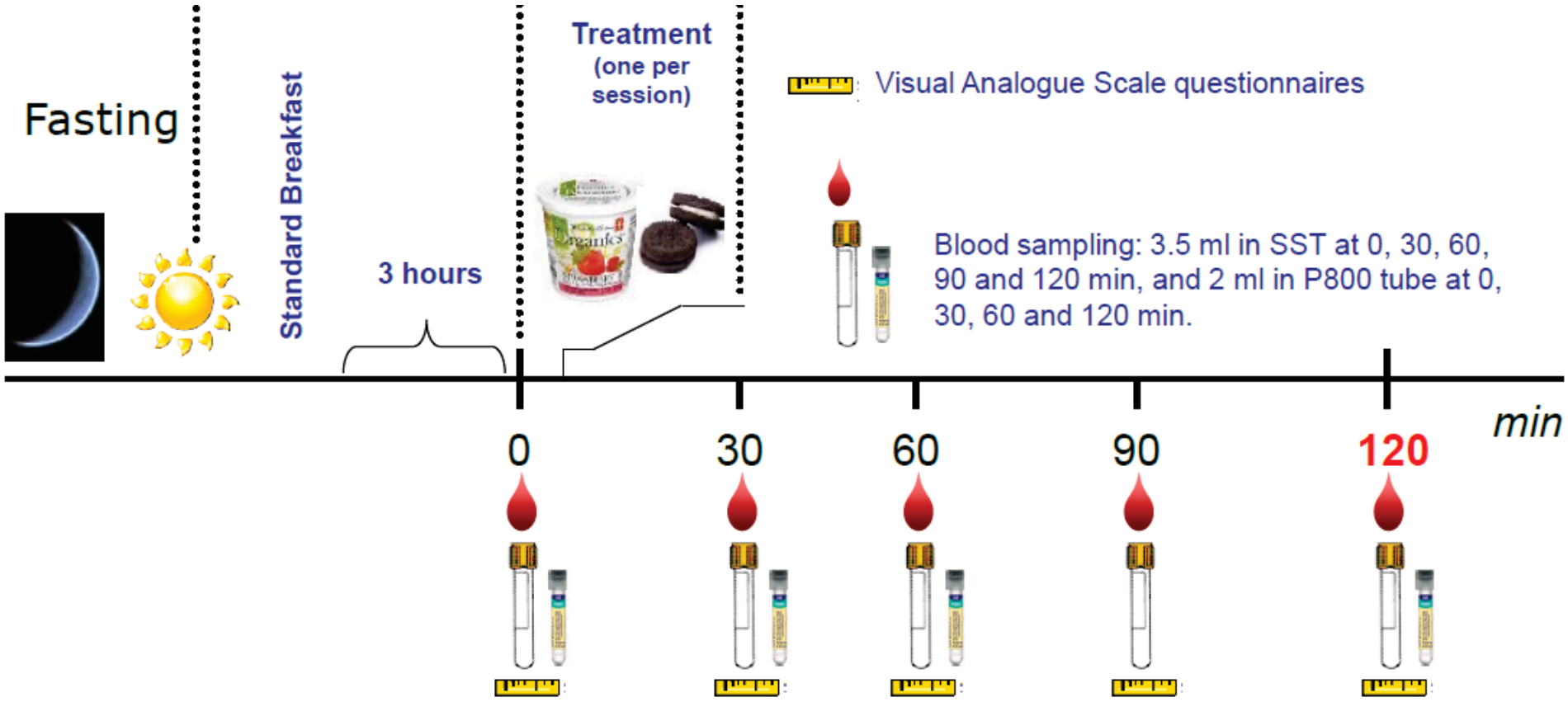
Energy and Macronutrients content per one treatment	*Yogurt (per 199 g)*	*Cookies (per 37.5 g)*
Energy (kcal)	*170.5*	*175.0*
Fat (total) (g)	*0*	*7.5*
Protein (g)	*17*	*1.3*
Total Carbohydrate (g)	*26.1*	*26.3*
Sugars (g)	*23.9*	*15.0*
Fibre (g)	*1.1*	*1.3*
Sodium (mg)	*62.5*	*212.5*
*Available carbohydrates (g)**	*25.0*	*25.0*

* Available carbohydrates were calculated as a difference between total carbohydrates and dietary fibre.

Appendix 13 Blood Collection System, Test Day Protocol, and Blood Sample Collection Protocol and Storage



Test Day Procedure



Protocol for blood collection in DFC study at IWK

1st tube to collect blood at 0, 30, 60, 90 and 120 min

BD Gold SST 3.5 ml tube, gently invert 5 times

→ Allow to clot for 30 min at room temperature

→ Spin at 1,000 – 1,300 g (RCF) for 10 min 4°C
****

→ Transfer 500 µL of serum in each of 3 microtubes (0.6 mL capacity), and the rest of the volume in the last (4th) microtube.
****ice bath****

Organize tubes in the boxes.

→

Store at -70°C

2nd tube to collect blood at 0, 30, 60 and 120 min

P800 2 ml tube, gently and slowly invert 8-10 times. Adjust to room temperature before use!

→ Spin at 1,000 – 1,300 g (RCF) for 10 min 4°C
****

→

****

Ice bath

→ Transfer 250 µL of plasma for GLP-1

Transfer 250 µL of plasma for CCK

Transfer 60 µL of plasma for PYY

Transfer 60 µL of plasma for Ghrelin into the tube with prior added 3 µL of 1N HCl. VORTEX!

Transfer 60 µL of plasma for Ghrelin (backup) into the tube with prior added 3 µL of 1N HCl. VORTEX!

Transfer 250 µL of plasma for "backup" (CCK or GLP-1)

Transfer 70 µL or whatever left: backup for all other analytes (incl. PYY and those unidentified at this moment)

→

Store at -70°C

Important! All SST, microtubes and boxes have to be labeled a day before the session. Please bring adhesive small pre-printed labels for P800.

Appendix 14 Ethics Approval

MOUNT SAINT VINCENT UNIVERSITY

Excellence • Innovation • Discovery

University Research Ethics Board

UNIVERSITY RESEARCH ETHICS BOARD

Certificate of Research Ethics Clearance

Title of project: *The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children*

Researcher(s): G. Harvey Anderson, Dr. Bohdan Luhovyy, Jill Hamilton, Nick Bellissimo

Supervisor (if applicable): n/a

Co-Investigators: n/a

File #: 2011-001

The University Research Ethics Board (UREB) has reviewed the above named proposal and confirms that it respects the *Tri-Council Policy Statement* as outlined in the *MSVU Policies and Procedures: Ethics Review of Research Involving Humans* regarding the ethics of research involving human participants.

This certificate of ethics clearance is valid one year from the date of issue. Renewals are available for up to two years in addition to the initial year and are contingent upon an annual submission to the UREB of a written request for renewal accompanied by a satisfactory annual ethics report thirty days prior to the expiry date as listed below. A final report is required within 30 days of expiry. Researchers are reminded that any changes to approved protocol must be reviewed and approved by the UREB prior to their implementation.

[redacted]

Dr. Michelle Eskritt, Chair
University Research Ethics Board (UREB)

October 19, 2011
Effective Date

[Expires: October 18, 2012]

MOUNT SAINT VINCENT UNIVERSITY

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University Research Ethics Board

UNIVERSITY RESEARCH ETHICS BOARD

Certificate of Research Ethics Clearance

File #:	**2012-063**
Title of project:	***The Effect of Dairy and Non-Dairy Snack Products on Glycemic Regulation in Normal Weight Children***
Researcher(s):	**Mary McCormick**
Supervisor (if applicable):	**Bohdan Luhovyy**
Co-Investigators:	**n/a**
Version :	**1**

The University Research Ethics Board (UREB) has reviewed the above named proposal and confirms that it respects the *Tri-Council Policy Statement* as outlined in the *MSVU Policies and Procedures: Ethics Review of Research Involving Humans* regarding the ethics of research involving human participants.

This certificate of approval is valid one year from the date of issue Renewals are available for up to four years in addition to the initial year and are contingent upon an annual submission to the UREB of a written request for renewal accompanied by a satisfactory annual ethics report thirty days prior to the expiry date as listed below. A final report is due on or before the expiry date. Researchers are reminded that any changes to approved protocol must be reviewed and approved by the UREB prior to their implementation.

[redacted] Dr. Daniel Séguin, Chair University Research Ethics Board (UREB)	**November 29, 2012** **Effective Date** ***[Expires: November 28, 2013]***

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Approval - Full Board Review
March 05, 2012

Principal Investigator: Dr Youness Anini
Title: The Effects of Milk Products on Glycemic Regulation, Satiety and Food Intake in Children. The Effect of Solid, Semi-Solid and Fluid Dairy Products on Blood Glucose, Food Intake and Satiety of Normal Weight and Overweight/Obese Children
Project #: 1008492
Meeting Date: 9/20/2011

The IWK Research Ethics Board (IWK-REB) has examined the application for this study. On behalf of the IWK-REB, I am pleased to confirm the Board's full approval for this research study, effective today. This includes approval for the following study documents:

Comments	Version Date
Protocol	2012/02/24
Research Summary	2011/09/06
Letter - Recruitment	2012/02/24
Information and Authorization Form	2012/02/24
Children's Assent Form	2012/02/24
Questionnaire - Telephone Screening	2012/02/24
Questionnaire - Puberty	2012/02/24
Questionnaire - Menstrual	2012/02/24
Questionnaire - Physical Activity	2012/02/24
Questionnaire - Dutch Eating Habits	2012/02/24
Visual Analogue Scale - Motivation to Eat	2012/02/24
Visual Analogue Scale - Pleasantness	2012/02/24
Visual Analogue Scale - Physical Comfort	2012/02/24
Advertisement	2012/02/24
Information Sheet - Blood Samples	2012/02/24

The Board's approval for this study will expire one year from the date of this letter (March 05, 2013). To ensure continuing approval, submit a Request for Continuing Review to the Board

2 - 4 weeks prior to the renewal date. If approval is not renewed prior to the anniversary date, the Board will close your file and you must cease all study activities immediately. To reactivate a study, you must submit a new Initial Submission (together with the usual fee, if applicable) to the IWK-REB and await notice of re-approval.

Please be sure to notify the Board of any of the following:

- Proposed changes to the initial submission (i.e. new or amended study documents)
- Additional information to be provided to study participants
- Material designed for advertisement or publication with a view to attracting participants
- Serious adverse events experience by local participants
- Unanticipated problems involving risks to participants or others
- Sponsor-provided safety information
- Additional Compensation available to participants
- Upcoming audits/inspections by a sponsor or regulatory authority
- Closure of the study (within 90 days of the event)

Approved studies may be subject to internal audit. Should your research be selected for audit, the Board will advise you and indicate any other requests at that time.

Important Instructions and Reminders

Submit all correspondence to Ethics Manager Bev White or Ethics Assistant, Joanne Leonard at the address listed at the top of this letter (do not send your response to the IWK-REB Chair or Co-Chair)

Be sure to reference the Board's assigned file number, 1008492 on all communications.

Highlight all changes on revised documents and remember to update version numbers and version dates, include a clean copy of all revised documents.

Best wishes for a successful study.

Yours truly,

[redacted]

Adam Huber
Co-Chair, Research Ethics Board

This statement is in lieu of Health Canada's Research Ethics Board Attestation: *The Research Ethics Board for the IWK Health Centre operates in accordance with:*
- Food and Drug Regulations, Division 5 "Drugs for Clinical Trials involving Human Subjects"
- The Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS(2)
- International Conference on Harmonization - Good Clinical Practice Guidelines - ICH-GCP

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Approval - Amendment
October 10, 2012

Principal Investigator: Dr Younes Anini
Title: The Effects of Milk Products on Glycemic Regulation, Satiety and Food Intake in Children. The Effect of Solid, Semi-Solid and Fluid Dairy Products on Blood Glucose, Food Intake and Satiety of Normal Weight and Overweight/Obese Children
Project #: 1008492

On behalf of the IWK Research Ethics Board (IWK-REB) I have examined the proposed amendment to this research study. I am pleased to confirm the Board's approval of the following amended documents, effective today:

Document Name	Version Date
Poster	2012/09/25

[redacted]
Adam Huber
Co-Chair, Research Ethics Board

The following is a complete list of approved documents for use on this study:

Document Name	Version Date
Protocol	2012/02/24
Research Summary	2011/09/06
Letter - Recruitment	2012/02/24
Information and Authorization Form	2012/02/24
Children's Assent Form	2012/02/24
Questionnaire - Telephone Screening	2012/02/24
Questionnaire - Puberty	2012/02/24

Questionnaire - Menstrual	2012/02/24
Questionnaire - Physical Activity	2012/02/24
Questionnaire - Dutch Eating Habits	2012/02/24
Visual Analogue Scale - Motivation to Eat	2012/02/24
Visual Analogue Scale - Pleasantness	2012/02/24
Visual Analogue Scale - Physical Comfort	2012/02/24
Advertisement	2012/02/24
Information Sheet - Blood Samples	2012/02/24
Poster	2012/09/25

This statement is in lieu of Health Canada's Research Ethics Board Attestation: *the Research Ethics Board for the IWK Health Centre operates in accordance with:*
- Food and Drug Regulations, Division 5 "Drugs for Clinical Trials involving Human Subjects"
- The Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans - TCPS (2)
- International Conference on Harmonization - Good Clinical Practice Guidelines - ICH-GCP