The Effect of Dairy and Non-dairy Snack Products on Glycemic Regulation in Over Weight and Obese Boys

By

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The rise in childhood obesity is associated with an increase in T2DM in children in Canada. An increase in snacking associated with higher energy and sugar intakes and a shift in consumption of traditional food groups have been identified as risk factors for both obesity and diabetes in the pediatric population. The objective of this study was to determine the effect of dairy and non-dairy snacks on glycemia in over weight and obese boys. Methods: In a repeated measures crossover design,
over weight (85^{th}-95^{th} BMI percentile) and obese (95th-99th BMI percentile) boys (n =7, age: 9-14 y) , were randomly assigned to consume one of two treatments: Greek-style sugar-sweetened yogurt (198.9 g, 171 kcal, 0 g fat, 26.1 g total carbohydrate, 23.9 g sugar, 1.1 g fibre, 17 g protein) and mini sandwich type cookies (37.5 g, 175 kcal, 7.5 g fat, 26.3 g total carbohydrate, 15 g sugar, 1.3 g fibre, 1.3 g protein). 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, insulin and C-Peptide at 0 min (immediately before the treatment), and at 30, 60, 90 and 120 min. Insulin secretion was calculated from deconvolution of plasma C-peptide and hepatic insulin extraction was calculated as mean C-peptide divided by mean insulin. Results: There was no difference between treatments in baseline glucose and insulin values. There was an effect of treatment ($P=0.05$) and time ($P<0.0001$) on glucose over 120 min but there was no time x treatment interaction ($P=0.3$). There was an effect of treatment ($P=0.0001$), time ($P<0.0001$) and a time x treatment interaction ($P<0.0001$) on insulin over 120 min. Mean two hour concentrations of glucose and insulin were 5% lower and 46% higher after the treatment with yogurt compared to the treatment with cookies, respectively. Pre-hepatic insulin secretion was not different between treatments ($P=0.017$) however, there was an effect of treatment ($P=0.001$) and a time x treatment interaction ($P<0.05$) on hepatic insulin extraction. At 30 min ($P<0.05$) and 60 min ($P=0.05$) the dairy snack resulted in lower hepatic insulin extraction compared to the non-dairy snack. The higher content of protein in the yogurt treatment (17 g) compared to the cookies treatment (1.3 g) may explain the observed difference between the treatments. Conclusion: In 9-14 y old over weight and obese boys a dairy snack reduced glycemia and increased circulating insulin levels by a reduction in hepatic insulin secretion 120 min after consumption compared to a non-dairy snack matched for available carbohydrate without affecting subjective appetite and subsequent food intake.

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List of Abbreviations

A

AAC = Area Above the Curve

ANOVA = Analysis of Variance

AUC = Area Under the Curve

B

BMI = Body Mass Index

C

CCfV = Canadian Center for Vaccinology

CCHS = Canadian Community Health Survey

CCK = Cholecystokinin

CHMS = Canadian Health Measures Survey

CVD = Cardiovascular Disease

F

FI = Food Intake

G

GI = Glycemic Index

GIP =Glucose-dependent Insulinotropic Peptide

GLP-1 = Glucagon-like Peptide 1

H

HDL = High density lipoprotein

HIE = Hepatic Insulin Extraction

I

iAUC = Incremental Area Under the Curve

IOTF = International Obesity Task Force

ISEC = Insulin Secretion

IWK = Izaak Walton Killam

M

MetS = Metabolic Syndrome

N

niAUC = Net Incremental Area Under the Curve

P

Peptide YY = PYY

S

SAS = Statistical Analysis Systems

SEM = Standard Error of the Mean

T

T2DM = Type 2 Diabetes Mellitus

tAUC = Total Area Under the Curve

V

VAS = VAS

W

WHO = World Health Organization

Chapter 1: Introduction

Childhood obesity in Canada has reached epidemic proportions, with 26% of children aged 2-17 years being over weight or obese (1). Nova Scotia, specifically, has the third highest rate of combined over weight and obesity (32%) of all provinces (1). Between 1978/79 and 2004 over weight and obesity increased from 12% to 18% and 3% to 8%, respectively in the 2- to 17-year old age range (1). Obesity is associated with an increased risk of chronic disease later in life including cardiovascular disease and T2DM mellitus (T2DM) (2). Furthermore, rates of T2DM in children and adolescents are rapidly increasing (3). In Canada, the average age of children diagnosed with T2DM is 13.7 years with 8% being younger than 10 years old and approximately 95% of children with T2DM are obese at diagnosis (4). The etiology of childhood obesity is complex and depends on a multitude of factors, such as the changing food supply and changing dietary patterns with increased consumption of highly processed foods and reduced consumption of traditional foods.

The increase in childhood obesity rates has been paralleled by a decline in dairy product consumption, therefore it is hypothesized that this decline is a causal factor in the increased prevalence of obesity and diabetes in children. Approximately half of Nova Scotian boys (48.6%) and girls (62.3%) in grade 7 eat fewer than the number of milk and milk product servings recommended in *Canada's Food Guide to Healthy Eating* (5). Frequent consumption of milk and milk products is associated with reduced energy intake and healthier body weights, and when milk products are consumed with carbohydrate it reduces hyperglycemia in adults (6-8). The short-term physiologic and metabolic effects of dairy products and their ingredients appear to provide and explanation for the healthier body weights and lower chronic disease rates reported in adults (8, 9). The relationship between dairy consumption, body weight, and glycemic control in children is currently unclear and the goal of this project is to elucidate how an existing relationship may be mediated.

Chapter 2: Literature Review

2.1 Childhood Obesity and Metabolic Disorders in Children

Short-term food intake (FI) and subjective appetite studies are being used in increasing frequency in children to determine the mechanisms by which different food and food components or environmental stimuli are contributing to childhood obesity (10-12). The same methodology can be used to determine how these same factors may be affecting glycemic regulation. Both questions are important as over weight and obesity as well as hyperglycemia after meals have been associated with the development of metabolic disorders such as cardiovascular disease (CVD) and diabetes (13).

Perturbations in glycemic control combined with obesity are early indicators of a progression towards T2DM mellitus (T2DM). The Canadian Diabetes Association has established criteria for conditions that present prior to the onset of T2DM including impaired fasting glucose (blood glucose values between 6.1 and 6.9 mmol/L following an 8 hour fast) and impaired glucose tolerance (two hour plasma glucose values between 7.8 and 11.0 mmol/L following an 75 g oral glucose challenge) (14). Interventions have been shown to improve the weight status and glycemic control of young children however more short-term experimental research can inform the best design of these interventions (15).

2.1.1 Obesity in Children

The most recent Canadian childhood over weight and obesity rates are estimated to be 19.8% and 11.7% respectively according to Canadian Health Measures Survey (CHMS) (16). This marks a decrease from the Canadian Community Healthy Survey (CCHS) (Cycle 2.2) rates in 2004 for over weight by approximately 6% and an increase in obesity by approximately 4% (17). However, a direct comparison between the two surveys is difficult because of the different ages included (2-18 compared to 5-17) and because the CHMS used the World Health Organization (WHO) growth charts to define over weight and obesity while the CCHS used the International Obesity Task Force (IOTF) guidelines. Additionally, the IOTF method has been shown previously to under

report over weight and obesity (18). Regardless, both reports show a high prevalence of over weight and obesity in Canadian youth.

High prevalence rates of over weight and obesity have deleterious consequences for the individual and society. The Public Health Agency of Canada and the Canadian Institute for Health Information estimated the annual economic burden of obesity, due to both direct costs to the health care system and indirect costs to productivity, in Canada rose by $735 million from $3.9 billion to $4.6 billion between 2000 and 2008 (19). Furthermore, poor diet quality of Canadian children in grade 5 has been shown to predict the frequency of access to health care indicating that the early financial ramifications that poor eating habits can have on the health care system (20). Obese children and adolescents have been shown to become obese adults (21). Adulthood obesity is a risk factor for coronary artery disease (22), hypertension, diabetes, dyslipidemia and the metabolic syndrome (MetS) (23).

2.1.2 Diabetes in Children

The Canadian Diabetes Association has identified two primary types of diabetes that can affect the pediatric population (24). Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder that results from β-cell destruction typically resulting in absolute insulin deficiency, manifesting in individuals who are genetically susceptible (25). In contrast, T2DM is the result of a progressive insulin secretory defect in a state of insulin resistance (26). Internationally, there has been a reported increase in the number of total diabetes diagnosis since 1980 (27).

The most recent, comprehensive diabetes surveillance report published by the Public Health Agency of Canada estimated that 2.4 million Canadians were living with diagnosed diabetes including 3000 children less than 19 years of age in 2008/09 (28). Furthermore, it is estimated that 450 000 cases of diabetes were undiagnosed at that time (28). In the United States T2DM accounts for about half of all cases of diabetes in the adolescent population and approximately one third of this group go undiagnosed (29). High levels of T2DM in adolescents are problematic because the earlier the onset the more difficult it is to control (30).

The etiology of T2DM is complex and can be a combination of *in utero* programming, genetic factors, catch up growth, poor diet quality, lack of physical activity and age (31). Adolescents presenting with type T2DM exhibit pancreatic β-cell dysfunction, high levels of central obesity, glucose intolerance, inflammation, and altered insulin sensitivity of the muscle, liver and adipose tissues (31). The difficulty of controlling diabetes in the pediatric population is due to its aggressive phenotype leading to a decline in β-cell function and more incidences of comorbidity (32, 33). Diabetes and obesity have both been identified as risk factors for the MetS, which is the clustering of health risk factors (34).

2.1.3 Metabolic Syndrome in Children

The MetS is the clustering of risk factors for noncommunicable diseases such as diabetes and CVD. In 2009 a joint statement by the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity was published, setting international standards for the definition of MetS (35). The presence of any three of the following criteria result in a positive diagnosis for MetS: 1) abdominal obesity (waist circumference ≥ 102 cm for males, ≥ 88 cm for females), 2) dyslipidemia (plasma triglycerides ≥ 1.7 mmol/L), 3) elevated fasting blood glucose (≥ 5.6 mmol/L), 4) decreased high density lipoprotein (HDL) (< 1.03 mmol/L) and, 5) elevated blood pressure (≥ 130/85 mmHg) (35). Data from the CHMS produced estimates that between 2009 and 2011 22% of Canadian adults (age 18-79 y) had MetS (36). Increasing levels of obesity and diabetes in children has led to more research in the pediatric population on the health outcomes of children who meet the MetS criteria.

Due to the physiological differences and instability in children and adolescence, particularly puberty associated insulin resistance; there is debate as to whether similar cut points can be used for measuring the risk of diabetes and CVD in the pediatric population (37). For instance, a study of 5235 children measured between the ages of 9-12 y and again at 15-16 y showed that waist circumference and fat mass (measured by dual-energy X-ray absorptiometry) was no better than body mass index (BMI) at predicting CVD risk

factors (38). Furthermore, an improvement in weight status, as measured by BMI, by obese children was associated with improved markers of insulin resistance including the baseline fasting insulin resistance index HOMA, and the components of MetS (15). Therefore, it can be concluded that BMI is an effective measure of central adiposity in children demonstrating the need for special consideration of metabolic risk factors in this group.

2.2 Food Consumption Patterns in Children

2.2.1 Eating Behaviours in Children

Food consumption patterns are subject to change based on environmental, lifestyle, economic and political variables and how the food industry reacts to changes in these variables (39). For example, in recent years there has been an increase in food prepared outside of the home (40) and the proportion of daily energy derived from snacks (41) while there has been a concurrent decrease in traditional food consumption (i.e. foods that can be classified in one of the four food groups in *Eating Well with Canada's Food Guide*). In the United States between 1965-1966 and 2007-2008 total energy intake in adults from home-sourced foods decreased between 24.5% and 23.9% while a concurrent increase in total daily energy intake from all sources rose between 118 kcal and 152 kcal (40). Children exhibit similar patterns. For example, from 1977 to 2006 total energy intake of children, aged 2-18 years, from food eaten outside the home increased from 23.4% to 33.9% paralleling an overall increase in energy of 179 kcal per day (42). It has been established that an increase in foods eaten outside of the home is associated with an increase in the amount of energy consumed however, during this time the patterns in which people eat, in particular children and adolescents, have also changed.

Eating between meals or snacking is a pattern that has become prevalent in the lives of children and depending on the content of the snack can influence weight status. Data from the 1977-1978 Nationwide Food Consumption Survey, and the 1989-1991 and 1994-1996 Continuing Survey of Food Intake by Individuals was used to compare children between 2 to 18 years of age and it was shown that snacking occasions have

increased while the caloric content of snacks has remained constant (41). In Canada, 63% of children between 4 to 18 years of age report eating snacks after school and that these snacks contribute 12-13% of their total daily energy intake (43). A possible explanation for the increase in snacking occasions is the ubiquity of prepared snack foods in children's environments. Systematic observation was used to analyze the availability and accessibility of snacks in 1082 stores in American metropolises found that energy-dense nutrient-poor snack foods were available in 96% of pharmacies, 94% of gas stations and between 29% and 65% of all other stores (44). With snacking an entrenched eating behavior a better of understanding of the implications of the behavior and what foods could be eaten as snacks to promote optimal health is necessary.

Establishing eating patterns in children and adolescents that will support healthy weight management and optimal growth and development is of primary importance. A longitudinal study that followed adolescents from age 14 to 21 reported that while dietary variation may be high week to week over time dietary habits tend to remain stable (45). Furthermore, a reduction in fruit and vegetable servings by an average of 0.7 from early to middle adolescence and an average of 0.6 serving from middle to late adolescence was reported (46). Additionally, using a longitudinal design, dairy consumption was shown to decrease between childhood (10 y old) and adulthood (19-28 y old) (47). The decline of traditional foods in early adolescence has been well documented and low intakes of these foods persist into adulthood highlighting the importance of establishing what the most beneficial foods for weight management and glycemic control are and ensuring children are eating them early and often in life.

2.2.2 Snacking Behavior

Evaluating the role of snacking in overall health is difficult because of the inconsistency in definitions among research protocols. In many study designs it is impossible to distinguish if eating behaviors should be properly classified as increased meal frequency or snacking. In previous studies snacks have been defined as intake of foods over a 15 min period and that were not included at a meal (41), food eaten outside of breakfast, lunch and dinner (48, 49), and others have allowed for the participants to define the term snack for themselves (50). Regardless of definition, it has been observed

that between 1977 and 2006 children living in the United States have increased their eating occasions from 3 to 5 per day (51). Furthermore, using data collected from the CCHS (cycle 2.2) it was found that 63% of all children and adolescents between the ages of 4 and 18 years report eating an afterschool snack (43). The changing eating patterns of children and adolescents have been well established however, the relationship between these patterns and weight status and glycemic control are less well understood.

Snacking can be hypothesized to contribute to Over weight and obesity attributed to increased energy intake because of the additional opportunities to consume energy as well as the poor food choices sometimes associated with snacks. Observational evidence supporting this association has been inconsistent. In a cross sectional study of 3267 adults using two nonconsecutive, multiple pass 24-h dietary recalls it was found that an increase in snack frequency was associated with greater energy intakes but not BMI (52). A study examining 24-hour recalls from 12 to 19 year old adolescents participating in the National Health and Nutrition Examination Survey (NHANES) 2001-2004 found that snacking frequency positively correlated with total energy and carbohydrate intake while negatively correlating with protein and fat intake (53). An increase in total energy intake in adolescents may indicate a greater BMI however, in this report this association was not found and increased snacking may simply be a method used by children with greater daily energy requirements to consume all of their necessary calories (53). Furthermore, even when snack foods were defined as food traditionally considered unhealthy such as, "sodas or other snack foods such as chips, chocolate bars, or cookies from vending machines at school," no association between the frequencies they were purchased and BMI in children was found (54). However, over weight and obese adolescents (14.3 ± 1.2 y) who underwent a weight loss intervention were less successful in keeping the weight off at a one year follow up if they snacked more often than the children who did not (55). Furthermore, a study of children aged 9 to 10 y found that there was no difference in the number of snacks consumed between children who were normal weight and those who had central adiposity (56). The normal weight children were found to eat snacks with lower energy density; higher carbohydrate content, higher fibre content and they were more likely to be fruits and vegetables (56). The inconsistency of evidence associating snacking with an increase in adiposity and the ubiquity of snacking behaviors

among youth suggest that research examining the most effective snacks for weight control is of merit.

Short-term, experimental studies underscore the importance of the type of the snack on energy balance and glycemic control. For example, a recent study in adults at risk for T2DM found that including 43 g/day of almonds as a snack for 4 weeks reduced serum postprandial glucose and did not cause an increase in daily energy intake (57). In children (age 8 to 11 y) when raisins or grapes were provided as a snack after school it was shown that cumulative FI (breakfast, morning snack, lunch and after school snack) was reduced compared with cookies and potato chips (11). Similarly, raisins as a snack reduced FI at a subsequent *ad libitum* test meal compared to grapes, a mix of raisins and almonds and water in 8–11 y old children offering further evidence that the composition of snack determines the physiological result (12). Short-term experimental research on the effect of snacking on FI and glycemic regulation is limited but shows that some traditional foods have beneficial effects, therefore more research on other types of traditional foods that may be eaten as snacks is required.

2.2.3 Dairy Consumption

Dairy foods including milk, cheese and yogurt currently contribute only 10% of total caloric intake for Americans while proving approximately 50% of dietary vitamin D and calcium intake (58). Additionally, the increase in childhood obesity rates have occurred at a time when dairy product consumption is in decline, therefore it is hypothesized that this decline is a causal factor in the increased prevalence of obesity and diabetes in children. Approximately half of Nova Scotian boys (48.6%) and two third of girls (62.3%) in grade 7 eat fewer than the number of milk and milk product servings recommended in *Canada's Food Guide to Healthy Eating* (5). A decline in dairy consumption among children and adolescents and the concurrent increased prevalence in obesity have led to observational and experimental investigation.

Frequent consumption of milk and milk products is associated with reduced energy intake and healthier body weights (6-8). Using data from NHANES III and a cross-sectional approach a study of 1803 adolescents aged 12 to 16 years found that children in the lowest quartile for waist circumference (a measure of central adiposity)

consumed nearly half a serving of dairy daily more than those in the highest quartile (59). These results are supported and furthered by prospective research using children from the Framingham Children's Study showing that girls and boys who consumed fewer than 1.25 and 1.70 servings of dairy respectively between the ages of 3 to 6 years gained an extra 25 mm of subcutaneous fat by early adolescence (60). When dairy is included in a hypocaloric diet of obese children the results are more favorable than a hypocaloric diet alone. After a three-year follow up of 120 obese prepubescent children, those who had lost weight on a dairy rich diet (>800 mg calcium/day) compared to an isolcaloric diet maintained a significantly lower waist circumference (61). The short-term physiologic and metabolic effects of dairy products and their ingredients appear to provide an explanation for the healthier body weights and lower chronic disease rates reported in adults (7, 9). However, the relationship between dairy consumption, body weight and glycemic control in children is unclear and requires more short-term experimental studies to establish a causal relationship.

2.3 Physiological Mechanisms of Blood Glucose Control and Food Intake Regulation

2.3.1 Glycemic Regulation

Glycemic regulation is a necessary homeostatic process that controls the amount of glucose in the blood. The process is regulated within a small range primarily due to the fact that the human brain depends uniquely on glucose for ATP production and therefore low levels of glucose, or hypoglycemia, can result in decreased functioning (62, 63). Furthermore, it is estimated that 40-80% of energy consumed daily is in the form of carbohydrate and thus will impact blood sugar levels (64). Furthermore, the Recommended Dietary Allowance for carbohydrate is 130 g which is necessary to meet the daily requirements of the central nervous system (65). Recent literature has highlighted the body's ability to detect and react to carbohydrate containing foods from their first contact with the mouth and beyond starting with the cephalic insulin response and terminating with nutrient interaction in the intestine (66). The many sites of glucose detection reflect the many overlapping mechanisms of glucose regulation within the

body, the most influential of which is insulin, counteracted by glucagon, epinephrine, cortisol and growth hormone.

Insulin is produced and secreted from pancreatic β-cells. The precursor of insulin, preproinsulin, quickly moves to the cisternal space of the rough endoplasmic reticulum after production where it is cleaved to the A and B chains of insulin connected by C-peptide. Since, C-peptide is cleaved to release insulin into circulation (67), C-peptide is secreted in a one-to-one molar ratio with insulin (68). Secretion of insulin occurs first during the cephalic phase of nutrient ingestion and then in response to the appearance of nutrients in the intestine (69). Ingested glucose increases intracellular islet glucose concentrations at which time glucose is transported into the β-cells primarily via GLUT2 (70). Insulin then promotes the uptake and storage of carbohydrate, fat and amino acids in skeletal muscle, adipose tissue and liver. An increase in circulating insulin also reduces hepatic glucose production by glycogenolysis and gluconeogenesis inhibition. After performing its action insulin is degraded and cleared from circulation.

Insulin is removed from the circulation primarily by the liver (~80%) where it is eventually degraded via lysosome activity with the remaining insulin being cleared by the kidneys and skeletal muscle (71). C-peptide is metabolized primarily by the kidney (85%) or excreted in the urine (72). To directly measure insulin secretion hepatic portal vein catheterization is necessary therefore, blood C-peptide levels provide a superior method of measuring insulin secretion. One of the major variables which requires measurement of C-peptide to accurately reflect insulin secretion is that hepatic insulin extraction is highly variable and can be reduced from 50% to 20% after ingestion of nutrients (73). Additionally, there is limited evidence suggesting that weight status contributes to hepatic insulin extraction with obese persons having lower basal levels and a larger decrease in response to food (74) however, when other metabolic markers are controlled for it appears as though obesity itself is not a strong enough predictor of insulin clearance (75). Insulin secretion itself is regulated in part by other endogenously produced peptides.

The presence of glucose in the intestine causes the release of hormones by the endocrine cells within the mucosa layer; the most important with regard to insulin dependent glucose regulation are the incretins (76, 77). There are two identified

incretins: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (78, 79). In addition to signaling insulin release, GLP-1 aids in the regulation of glucose by inhibiting glucagon secretion and gastric emptying via the ileal brake (80). The primary site of intestinal production of GLP-1 occurs in the enteroendocrine L cells in the distal small intestine however GLP-1 levels have been observed to rise soon after eating suggesting that secretion is stimulated before glucose reaches these L cells (81). In contrast, GIP molecules are synthesized in enteroendocrine K cells in the proximal small intestine but exert similar effects once released (79). Once the β-cells have been signaled to release insulin they continue until blood glucose disposal is complete and basal levels are restored. During a fasted state, glycemic levels are regulated via the release of glucose, primarily from the liver, through the secretion of glucagon (77). The amount of glucose released by the liver is directly related to the amount of mass occupied by metabolically active tissues reiterating the notion that glucose in the blood is tightly regulated due the importance of it as a metabolic energy source (82). Glucose regulation has also been hypothesized to play an integral role in FI regulation.

2.3.2 Glycemic Regulation and Physiological Control of Food Intake

The regulation of FI and appetite via changes in blood glucose concentrations was first hypothesized by Jean Mayer in the early 1950s (83, 84). He hypothesized that a decrease in blood glucose utilization, representative of available glucose, would precipitate feeding and a rise in utilization would inhibit feeding (83, 84). This reduction in blood glucose was posited to be detected by sensory cells in the ventral hypothalamus (85) suggesting that the availability of glucose to the brain, due do its small capacity for glucose storage, was the most important factor mediating FI. This hypothesis was supported by the administration of 2-deoxyglucose, a drug that interferes with normal glucose metabolism, to monkeys and rats resulting in increased eating (86). While glucoprivation, or drug disrupted glucose metabolism, shows that low glucose levels will initiate feeding these findings cannot necessarily be extrapolated to humans in free-living conditions, as normal physiological blood glucose variations are not as extreme as those induced experimentally. Using Mayer's glucostatic theory as a base, foods with a high

glycemic index (GI) have been hypothesized to cause greater subsequent FI and lead to an accumulation of excess of body weight.

The GI is a measure of how much glucose can be found in the blood after eating and how long this level is sustained (87). The GI of a given food is determined by providing a food, typically containing 50 g CHO, and measuring blood glucose concentrations over a predetermined amount of time and then comparing the results to a reference food (with an arbitrary GI score of 100) (87). The GI has several limitations that have been discussed in length in the literature such as its lack of consideration for processing, presence of other macronutrients (88), amounts commonly eaten, and poor intersubject reliability (89). It has been shown experimentally that obese boys (age 15.7 ± 1.4 y) consume more food at a subsequent meal after a meal classified as high-GI compared to a medium (53%) or low (81%) and that 53% of the variance in FI within participants was attributable to the area under the glycemic response curve after the first meal (90). These results appear to confirm that a high GI meal will cause an increase in blood insulin levels resulting in lower postabsorptive blood glucose levels leading to greater energy intake at the next meal however, in this study the meals differed in macronutrient composition and grams of fibre was not reported making direct interpretation difficult (90). It has also been shown the GI of a food may not effectively predict the insulin response, for example when adults (22-30 years) consumed 16.2 g of whey protein the insulin response was similar to white bread however the area under the curve (AUC) blood glucose was significantly lower (91). Furthermore, whole milk products have been shown to have insulinemic indexes 3 to 6 times greater than predicted based on their corresponding GI (92). However, it can be concluded that the amount of glucose in the blood is an effective indicator of subsequent FI and the expected satiety response.

Satiety is defined as the inhibition of hunger and further eating as a result of previous food consumption and satiation is the process that brings a meal to a close (93, 94). The satiating efficiency of a food refers to a consumed food's ability to suppress hunger and to inhibit the onset of a further period of eating (93). Satiation signals refer to hormones, chemicals and interactions of the enteric nervous system that influence FI and

the feeling of fullness in humans (95). Satiety and satiation are hormonally regulated by satiation signals and adiposity signals (96).

Satiation signals generally possess similar characteristics, they are endogenously produced, are secreted in response to FI, act within a single period of eating, reduce FI without causing illness, and FI is increased when their activity is blocked within the body (97). Known anorexigenic satiation signals include cholecystokinin (CCK), GLP-1, and peptide YY (PYY) among others. The only known peripheral hormone that exerts an orexigenic effect is ghrelin (96, 98, 99). Adiposity signals such as leptin and insulin are synthesized and secreted in amounts proportional to the total amount of body fat and function to regulate energy stores over time rather than FI within a specific meal (100). Peripherally secreted satiation signals and adiposity signals affect FI and maintain constant body weight by their action at the arcuate nucleus of the hypothalamus (101). These peripherally secreted peptides pass through the blood brain barrier to interact with neurons resulting in the production of pro-opiomelanocortin, neuropeptide Y and agouti-related peptide that send signals to the paraventricular nuclei which reduces FI or the lateral hypothalamic area which increases FI (102, 103). The obese state is evidence that this system is prone to failure and therefore an examination of the physiological differences between obese and lean people is necessary.

2.3.3 Glycemic Regulation and Physiological Control of Food Intake in the Obese State

The obese state is characterized by increased levels of some anorexigenic hormones which theoretically should reduce FI eventually favouring the return to a normal body weight however this system seems to be ineffective in most instances. For example, the use of recombinant leptin therapy has been successfully employed to reduce body weight, up to 54% in children and adults with identified leptin deficiency (104). However recombinant leptin therapy was only shown to cause weight loss in some non-leptin deficient obese participants and this weight loss was minimal after 24 weeks (105) proving that low circulating levels of leptin are insufficient to characterize obesity.

Unlike leptin, blood levels of insulin are far more dependent on ingested nutrients with approximately 50% of total insulin secretion being in response to feeding (98, 100). The obese have higher levels of circulating insulin compared to their normal weight

counterparts despite a similar secretory pattern (100). The typical pattern of insulin secretion is pulsatile with 80% of those pulses coming in response to an increase in plasma glucose concentration due to feeding (100). It is paradoxical that leptin and insulin are higher in the non-genetically obese however resistance can explain this.

Insulin resistance is identified as a dysregulation of the suppression of endogenous glucose production and when glucose uptake by insulin dependent tissues is impaired and therefore more insulin is produced by pancreatic β-cells it is thought to lead to hyperinsulinemia and eventually T2DM (106). Additionally, the obese and insulin resistant person exhibits greater levels of inflammation markers, particularly white blood cells and increased levels of pro-inflammatory cytokines, originating in the adipose tissue and liver, entering the blood stream and resulting in systemic inflammation (107). The increase of inflammation associated with obesity has been attributed to multiple factors including ER stress, adiponectin reduction, leptin elevation, adipocyte death, macrophage infiltration and lipolysis (107). The obese and T2DM possess similar physiological profiles including increased inflammation, insulin resistance, and attenuated incretin response however research in humans has shown that these profiles can exist independently (108) and highlight the necessity of research on obese individuals who have not been diagnosed with T2DM.

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

Observational evidence has linked an increase in dairy intake with a decrease in obesity, an improvement in glycemic control and reduction in inflammation (59-61, 109, 110). The components in dairy that have been hypothesized to be responsible for this association include macronutrient composition (8), calcium (111), and specific bioactive peptides (112).

Epidemiological studies associating dairy with healthier body weights have been supported by short-term experimental studies on appetite and satiety that show the macronutrient composition, particularly the high protein content, of milk can explain these effects. The two major proteins in milk are whey (80%) and casein (20%) (113)

both of which are complete proteins and are comparatively high in branched chain amino acids (114). Whey and casein proteins have been characterized as fast and slow proteins respectively due to their rate of digestion (115). A study in healthy, male adults showed that giving 20 g of casein but not whey 30 min before a test meal decreased *ad libitum* FI compared to water and increased subjective ratings of appetite although, cumulative FI (drink kcal + meal kcal) did not differ (116). However, the whey condition was shown to significantly lower blood glucose compared to 20 g of protein from casein or pea protein and water (116). In type 2 diabetics when 55 g of whey was provided as a preload 30 min before a potato meal, blood glucose, expressed as AUC, was reduced and insulin, GIP, GLP-1, and CCK increased showing that dairy proteins can aid in glycemic control and increase the secretion of satiation signals (117). An increase in anorexigenic satiation signals and a decrease in ghrelin have also been observed in non-diabetic lean and obese men (118, 119). In boys (age 9-14 y), whey protein (1.0 g/kg) 30 min before an *ad libitum* test meal was shown to reduce FI in normal weight but not over weight participants (10).

Some randomized control trials have evaluated the effects of dairy products on inflammation in adults. For example, over weight and obese adults with MetS that consumed more than 3.5 servings of dairy daily for 12 weeks had decreased measures of inflammation, including proinflammatory cytokines and increased adiponectin compared to over weight and obese adults on an isocaloric diet consuming fewer than 0.5 servings of dairy daily (120). Furthermore, the 3.5 servings of dairy group reduced their level of adipose tissue without a change in weight whereas no significant differences were found in inflammatory markers, body weight or adiposity in the lower dairy group (120). Similarly, Zemel and Sun found that when 39 obese African American adults consumed isocaloric diets with either 3 daily servings of dairy or 1 daily serving of dairy there was an increase in adiponectin and a reduction in C-reactive protein, a marker of inflammation, in the high dairy group in addition to a reduction in waist circumference, trunk fat and body fat (121). Animal research has shown that calcium may be responsible for the reduction of inflammatory cytokine expression in adipocytes (122) as well as the ability of certain dairy bioactive peptides to regulate cytokine releasing immune cells (123). The research to date supports the use of dairy products to help suppress the

inflammation associated with obesity however, a lack of studies showing inflammation reduction independent of improving body composition make determining causation difficult.

To date there is currently a lack of research elucidating the relationship between eating behaviours, such as snacking, and traditional foods, such as dairy, on food consumption, body weight, and glycemic control in over weight and obese children. The increase in energy consumption associated with increased snacking occasions is often associated with a high intake of available carbohydrates. Therefore, it is important to study how the various components of different snack foods, particularly macronutrients, may impact the components of the glucose regulating system in children.

Chapter 3: Rationale and Objectives

3.1 Rationale

Currently, children do not meet the recommended intake of milk and milk products and they over consume sweet and salty snacks. This experiment will be one of the first of its kind (short-term experimental) to investigate if a causal relationship between dairy product consumption and healthier glycemic response and subsequent FI in overweight and obese children exists. This research will also elucidate the metabolic and hormonal mechanisms mediating the relationship.

3.2 Objectives

1) To investigate the postprandial glycemic response to a dairy and non-dairy snack within two hours.
2) To investigate the effect of consuming a dairy and non-dairy snack on the insulin response within two hours.
3) To evaluate the effect of a dairy snack with a non-dairy snack consumed two hours before a test meal on FI and subjective appetite pre and post an *ad libitum* test meal.

3.3 Hypotheses

1) A dairy snack (yogurt) will affect post-prandial glycemic control via a decrease in blood glucose and an increase in insulin secretion compared to a non-dairy snack food in over weight and obese 9-14 year old boys.
2) A dairy product provided as a snack 120 min before an *ad libitum* test meal will reduce subsequent food intake and increase satiety in 9-14 year old over weight and obese boys compared with a non-dairy snack.

Chapter 4: Methods

4.1 Study Design

A within-subjects randomized crossover design was employed to examine the effect of a dairy snack and non-dairy snack food on glycemic regulation, FI regulation and subjective appetite. On each test day the participants received one of two treatments that had been matched for available carbohydrate: (a) Greek yogurt or (b) sandwich type cookies. The participants were randomly assigned a treatment order so that the experiment was counterbalanced. Blood samples were taken at 30 min intervals five times throughout the study for insulin and glucose analysis. An *ad libitum* lunch was offered 120 min after either treatment and subjective appetite was measured throughout the duration of the sessions.

4.2 Participants

All participants recruited were 9-14 year old over weight or obese boys. Over weight and obese boys were defined by age- and sex- specific BMI percentiles according to the Canadian interpretation (124), at the time of study design, of the CDC growth charts (125). Children who were $\geq 85^{th}$ percentile of BMI-for age and $< 95^{th}$ percentile were considered over weight and $\geq 95^{th}$ percentile were considered obese. Children were excluded from the study if they had food sensitivities or allergies, did not regularly consume breakfast, were unable to tolerate the treatments, were not born at term ($\geq$37 and $\leq$42), were taking any medications or had any condition which may have affected appetite or glucose regulation, or if they had emotional, developmental and learning problems. An anticipated sample size of 8 children was recruited based on previous work in normal weight children and a sample size calculation showing that 7 participants would be sufficient to detect differences in blood insulin levels in response to the treatments provided in the current study (**Appendix 1**).

Participants from the Halifax Regional Municipality were recruited via the Izaak Walton Killam (IWK) Canadian Center for Vaccinology (CCfV) participant database, IWK Facebook page, MSVU Appetite Lab database, on-line advertisements (Kijiji, HRM parent website, etc.), flyer distribution (**Appendix 2**) and by word of mouth. Parents who

expressed interest in their child participating in the study were contacted electronically (e-mail) or by phone for the first stage of a two-stage screening process. The first stage provided the child and their guardian with details about the experiment and determined if the potential participant was eligible for the study (**Appendix 3**). Children were then invited to MSVU for the second stage of the screening process.

During part two of the screening process all parameters of the study were explained in detail and all questions addressed, after which written consent (**Appendix 4**) was obtained from the guardian and written assent from the boy (**Appendix 5**). Age and physical measures including height in metres (using a stadiometer), weight in kilograms (using an electronic scale) , and body composition based on foot-to-foot bioelectrical impedance analysis (BIA) via a Tanita Body Composition Analyzer (Tanita TBF-300A; Tanita Corporation of America, Inc, Arlington Heights, IL), were taken. All participants were made aware that the machine operates by sending a small undetectable electrical impulse through their body. Potential participants also completed a Physical Activity Questionnaire (**Appendix 6**).

A self-administered Tanner Staging (**Appendix 7**) and puberty questionnaire **(Appendix 8**) measured what stage of pubertal growth the participant was at because of the hormonal changes that occur during this time particularly the transient insulin resistance associated with an increase in growth hormones, sex hormones, and insulin-like growth factors (37). Due to the sensitive nature of these questionnaires they were first presented to the guardian who had the option of completing them or allowing their child to complete it.

The Dutch Eating Behavior Questionnaire (DEBQ) (**Appendix 9**) was administered during the second stage of the screening process to determine if any of the participants exhibited high levels of dietary restraint, emotional, or external eating behaviors. The original DEBQ (126) was developed by Van Strein and colleagues and consisted of 33 questions with 5 potential answers. The original DEBQ was adapted in 2008 for a younger population (127). The version used in this study contained 20 questions that had the possible answers, “Yes,” “Sometimes,” and “No.”

4.3 Session Protocol

The research took place at the CCfV at the IWK Health Centre in Halifax, NS. The experiment consisted of two sessions, on two separate days but at the same time, one week apart where children randomly received either a dairy or non-dairy dietary treatment and blood samples were collected over the next two hours. The treatments were: (a) Strawberry low fat yogurt (Liberté, St. Hubert, Canada), and (b) Mini Oreo Mr. Christie's cookies (Kraft Canada). Both treatments had 25 g of available carbohydrates. Children arrived at the IWK at 9:30 AM or 10:00 AM (but consistent throughout), after eating a standardized breakfast provided by research assistants, that was consumed at home and broke a 12 hour fast (except for water). Upon arrival children completed visual analogue scales (VAS) assessing subjective appetite following a baseline blood sample taken by a registered nurse. After collecting the baseline sample children consumed the dietary treatment at their regular pace. The pleasantness of the treatment was assessed using a VAS questionnaire. Blood samples for glucose and insulin occurred at 0, 30, 60, 90 and 120 min. Before each blood sample VAS assessing physical comfort and subjective appetite was administered. All blood samples were aliquoted and frozen at -80°C until analysis.

4.3.1 Dietary Treatments

Participants received, in random order, both the dairy and sandwich type cookie snack over the course of two test day sessions. The dairy snack was Organic, low fat, strawberry Greek yogurt (Liberté, St. Hubert, Canada) and the sandwich type cookie snack was Mini Oreo Mr. Christie's cookies (Kraft Canada, Don Mills, ON). The two treatments each contained 25 g of available carbohydrate (**Appendix 10**). Each participant was served 199 g of yogurt containing 170.5 kcal, 25 g carbohydrate, 0 g fat and 17 g protein. *Canada's Food Guide* states that one serving size of yogurt is 175 g (3/4 cup) therefore the amount of yogurt in each treatment (199 g). Each sandwich type cookie snack treatment weighed 37.5 g and contained 170 kcal, 25 g carbohydrate, 7.5 g fat and 1.3 g protein. The size of each treatment was chosen to replicate an amount of food typically eaten as a snack. Both treatments were prepared on session days, prior to

the participants' arrival. A time of 8 min was allotted for participants to consume the treatments in their entirety at a normal pace.

4.3.2 Blood Collection Protocol

Blood samples were taken from the participants, on both test days, at 0, 30, 60, 90 and 120 min. A registered nurse that was licensed to practice in Nova Scotia performed all blood collections. To ensure safety all nurses wore non-latex gloves and used aseptic technique when drawing blood. Non-latex gloves were used as latex has been identified as an allergen for many in the general population and to reduce the chance of direct contact with blood specimens. Over the course of 120 min 3.5 mL of blood was extracted.

A shielded intravenous catheter (BD Insyte Autogaurd™) connected to a Luer-Access split septum device (BD Q-Syte™) and Luer-Lok™ access device (BD Vacutainer®) (**Appendix 11**) system was used to collect the blood samples. This system was chosen to reduce the risk of bloodstream infection and because it provided a greater flow rate. The catheter and split septum device remained attached to the participant for the duration of the blood draws (120 min) however, the access device was discarded after each draw. Saline was flushed through the split septum device after each draw to eliminate syringe-induced blood reflux. Samples were collected in BD 3.5 mL Vacutainer® Plus (Becton, Dickinson and Company, Mississauga, ON) plastic serum separating tubes (SST). These specific SST tubes contained a clot activator and serum gel separator, which aided in preparing the samples for glucose, insulin and C-peptide analysis. Serum separating tubes were attached to the catheter so specimens were collected at 0, 30, 60, 90 and 120 min (**Appendix 12**).

Immediately before a blood draw took place, stickers with the date, time, time point, treatment ID and participant ID were adhered to the SST tubes. Immediately after a blood draw took place the SST tube were gently inverted by hand five times and then set at room temperature for 30 min prior to centrifugation. The centrifuge spun at 1,300 g relative centrifugal force (RCF) for 10 min at 4^{o}C. Immediately after 10 min, the SST tube was removed from the centrifuge and aliquoted into 0.6 mL capacity micotubes labeled with the following information: date, time, time point, treatment ID and

participant ID. Two micotubes received 500 µL of sample (for glucose analysis) and two received 150 µL of sample (for insulin analysis). Once the entire sample had been aliquoted to the appropriate microtube they were placed in previously labeled boxes and stored in a -80^{o}C freezer (**Appendix 13**).

4.3.3 Food Intake

Macaroni and cheese (Kraft Dinner *Easy Mac*, Kraft Canada, Don Mills, ON) was used as the test meal because of its uniform energy and macronutrient composition as well as its familiarity and acceptance with children in this age range. Three bowls were served at 10 min intervals. Once the macaroni and cheese was cooked it was weighed (g) using an analytical balance, recorded and served to the subjects. At the end of each 10 min interval the remaining macaroni and cheese was weighed and subtracted from the initial weight to determine the amount of macaroni and cheese consumed. The values obtained in grams were converted to energy (kcal) values using the manufacturer's information. A bottle of water was also provided and was weighed before and after the test meal. Participants were asked to eat until they were "comfortably full."

4.3.4 Subjective Appetite

The VAS were used to measure all subjective variables throughout the study including average appetite (AA), physical comfort (PC), sweetness and palatability of the treatment and pleasantness of the test meal. The scales presented a question such as, "how hungry do you feel?" and below a 100 mm line appeared with opposing answers to the question such as "not hungry at all" and "as hungry as I have ever felt" anchoring either end. The participant was then instructed to make a mark that intersected the line vertically indicating where along the continuum best represented their internal state. The mark was then measured from the left side of the line and this value was the individual's score (**Appendix 14**). This method of determining subjective appetite had been used previously in studies in both adults and children (128, 129) and was intended to provide a multi-factorial representation of hunger (93). Average appetite represents four different dimensions (Desire to eat [DTE], Hunger, Fullness and Prospective Food Consumption [PFC]) of subjective appetite (130) and had been used previously in this age group (10, 128, 131-134). The participants are presented with a piece of paper asking four questions

that represent DTE, hunger, fullness and PFC respectively: 1) "How strong is your desire to eat?" ("Very weak" to "Very strong"), 2) How hungry do you feel? ("Not hungry at all" to "As hungry as I have ever felt"), 3) How full do you feel? ("Not full at all" to "Very full") and 4) How much do you think you can eat? ("Nothing at all" to "A large amount"). This VAS scale was administered at 0, 30, 60, 90, 120 and 150 min (**Appendix 15**). Average appetite was calculated by using the four subjective appetite scores for each time point using the formula:

AA score (mm) = [DTE + hunger + (100-fullness) + PFC] / 4 (10, 128, 131-135).

The reproducibility of VAS has been investigated in adults and mean 4.5 hour appetite measures were found to be the most reproducible however, post-meal VAS scores were more strongly correlated with intake at a test meal than pre meal values (136). Other studies have shown reproducibility among adults to be high when taken by the same participant immediately after each other (137) but not by the same participant on different days (138). The use of 100 mm and 150 mm VAS have been shown not to differ from each other, when a variety of VAS surrounding appetite were tested in both lengths the correlation coefficients were found to be high r=0.80-0.98 ($p < 0.01$) (139). Furthermore, it has been shown that composite AA scores as determined by VAS are highly reproducible prior to a test meal (133). In this study there was a great deal of variation in baseline VAS measures when the subjects first arrived at the laboratory despite a standardization of nutrient intake after waking. This finding is in agreement with adult data and may simply be a function of biological variation and not methodological limitation however at present it is impossible to distinguish between the two.

4.3.5 Subjective Sweetness of the Treatments

The subjective perception of the sweetness of the treatments was evaluated at 8 min using the following question: "How sweet have you found the snack?" ("Not at all pleasant to "Very Pleasant") (**Appendix 16)**.

4.3.6 Subjective Palatability of the Treatments

The perceived palatability of the treatments was determined by presenting the boys with a VAS scale at 8 min asking the question: "How sweet have you found the snack?" ("Not sweet at all" to "Extremely sweet") (**Appendix 16**).

4.3.7 Subjective Physical Comfort

Physical comfort was measured at 0, 8, 30, 60, 90, 120 min and finally after the test meal (150 min) using a VAS scale asking the question: "How well do you feel?" ("Not well at all" to "Very well) (**Appendix 17)**.

4.3.8 Blood Glucose, Insulin and C-Peptide Analysis

Blood glucose, insulin and C-peptide samples were collected and analyzed at five time points throughout the study (0, 30, 60, 90 and 120 min). Blood glucose levels were determined in blood serum. A YSI 2300 STAT Plus Glucose Analyzer (YSI Life Sciences Yellow Springs, OH, USA) will be used for glucose analysis. The YSI machine uses enzyme sensor technology to produce hydrogen peroxide that is oxidized to produce free electrons; their flow is directly proportional to the peroxide concentration and to the concentration of the substrate.

Samples for blood insulin were prepared in the same method as blood glucose samples. A sandwich type enzyme-linked immunoabsorbent assay (ELISA) was used for serum insulin and C-Peptide analysis. The ELISA assays use 96 well microplates that are pretreated with monoclonal antibodies specific for insulin or C-peptide. The concentration of analytes and the quality of assays were controlled using insulin or C-peptide standards and diabetes controls which are human serum samples with known concentrations of insulin or C-peptide. Samples with unknown amounts of insulin or C-peptide will be pipetted into individual wells on the microplate for an initial incubation after which a stop solution is applied to terminate the reaction. A spectrophotometer was used to measure the optical density of each well; the optical density is a direct representation of the concentration of insulin or C-peptide in the well.

Blood C-peptide was used to calculate pre-hepatic insulin secretion rate (ISEC). This calculation involves the deconvolution of plasma C-peptide concentrations which

must account for the disruption of C-peptide's linear kinetics in non-steady-state conditions (140). Deconvolution is necessary because when C-peptide is measured the value reflects not only that which is being secreted but also that which was previously secreted and results in a cumulative measure (141). Therefore, insulin secretion software was developed to account for the disappearance rate of C-peptide based on population data that showed that C-peptide disappearance can be predicted based on weight, height, age, sex and weight status (141, 142).

4.4 Data Analysis

Statistical Analysis Systems (SAS) version 9.2 (SAS Institute Inc., Carey, NC, USA) was used for all statistical analyses with all data reported as mean ± SEM (standard error of the mean). Hepatic insulin extraction (HIE) was calculated by dividing mean C-peptide levels by mean insulin levels. The division of C-peptide levels by insulin levels is intended to represent HIE as a large proportion of insulin is removed by the liver during the first circulation and C-peptide is not therefore, it can be better understood why differences in insulin secretion are or are not apparent. The ISEC for each subject was calculated by the deconvolution of plasma C-peptide concentrations using the ISEC software package developed and provided by Hovorka (141) Net incremental AUC for all biochemical measures, and net area above the curve (AACs) for subjective appetite was calculated for 0-120 min. Three-way repeated measures ANOVA was used to determine the effects of treatments, time and the time-by-treatment interaction for all biochemical measures and average appetite scores over the duration of the experiment followed by a two-way repeated measures ANOVA to determine the effects of treatment at the specific time points. The effect of treatments on FI and on biochemical measures AUCs and average appetite AAC were determined by two-way repeated measures ANOVA. *Post hoc* analysis using Tukey-Kramer's test was performed when the significant treatment or interaction effect as indicated by $p = 0.05$ was found. Correlations on dependent measures were conducted using Pearson correlation coefficients.

Chapter 5: Results

5.1 Baseline Participant Characteristics

Seven boys aged 9- to 14-years-old with a BMI percentile between the 85th and 99th percentile range specific for age and gender participated in the study. All baseline characteristics of the participants are listed in **Table 5.1**.

Table 5.1 Baseline Characteristics for All Study Participants

Subject Characteristic	All (n=7)
Age (years)	11.1 ± 0.6
BW (kg)	54.8 ± 5.8
Height (m)	1.52 ± 0.06
BMI (kg/m^2)	23.8 ± 1.1
BMI %ile	93.7 ± 1.4
FM (kg)	16.9 ± 2.2
FFM (kg)	40.0 ± 4.0
TBW (kg)	29.3 ± 2.9
DEBQ Average	1.6 ± 0.2
External Eating Score	2.0 ± 0.2
Restrained Eating Score	1.6 ± 0.2
Emotional Eating Score	1.3 ± 0.1
Tanner Stage	1.6 ± 0.4
PA Hours Per Week	6.6 ± 1.4
PA METS Per Week	34.0 ± 6.3

Data are means ± SEM, n = 7. 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 Study Participants

Tanner Stage	Number of Participants
Stage 1	5
Stage 2	1
Stage 3	0
Stage 4	1

5.2 The Composition of Dietary Treatments

The two treatments were (a) Strawberry organic Greek yogurt (Liberté, St. Hubert, QC) and (b) Mini Oreo Mr. Christie's cookies (Kraft Canada, Don Mills, ON). 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**). Perceived sweetness ($P = 0.96$) and perceived pleasantness ($P = 0.09$) was not different between treatments **(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 carbohydrate (g)[b]	25.0	25.0
Sweetness (mm)	62 ± 10	56 ± 14
Treatment Pleasantness (mm)	51 ± 14	74 ± 6

Data are means ± SEM, n = 7.

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

5.3 Blood Glucose

There was an effect of treatment (P=0.05), time (P< 0.0001), but no treatment x time interaction (P=0.31) on mean cumulative blood glucose concentrations **(Table 5.4)**. There was an effect of treatment (P<0.05) and time (P<0.001), but no treatment x time interaction (P=0.38) on mean cumulative blood glucose concentrations when expressed as change from baseline. Both mean blood glucose levels and change from baseline blood glucose scores were reduced following the dairy treatment when compared with the cookie treatment however, because there was no interaction of treatment x time this was not attributable to differences at any given time point **(Figure 5.1)**.

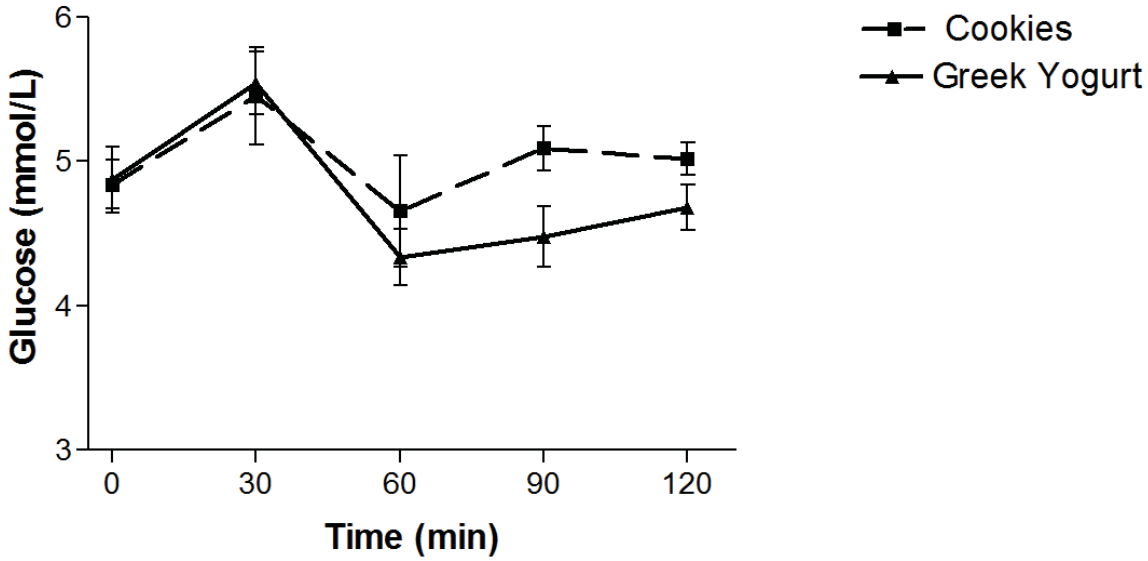
There was a trend indicating blood glucose total area under the curve (tAUC) was lower after the dairy treatment compare to the cookies treatment (P=0.08). There was an effect of treatment on incremental area under the curve (iAUC) (P<0.05) and net incremental area under the curve (niAUC) (P<0.05) **(Table 5.4)**. After the dairy treatment iAUC and niAUC were lower compared with the cookies treatment.

Table 5.4 Blood Glucose Response over 120 minutes

	Treatments	
	Greek Yogurt	Cookies
Mean Blood Glucose (mmol/L)	$4.8 \pm 0.1^{1,2}$	$5.0 \pm 0.1^{1,2}$
Mean Blood Glucose Change from Baseline (mmol/L)	$-0.1 \pm 0.1^{1,2}$	$0.2 \pm 0.1^{1,2}$
Glucose tAUC (mmoL • min/L)	573.9 ± 18.3	603.9 ± 22.0
Glucose niAUC (mmoL • min/L)	-10.9 ± 18.0^{1}	22.9 ± 11.4^{1}
Glucose iAUC (mmoL • min/L)	21.0 ± 6.6^{1}	34.6 ± 8.5^{1}

Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1]an effect of treatment; [2]an effect of time. P<0.05.

Figure 5.1 Mean Blood Glucose Concentrations over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.

5.4 Blood Insulin

There was an effect of treatment (P=0.0001), time (P<0.0001), and a treatment x time interaction (P<0.0001) on mean cumulative blood insulin concentrations **(Table 5.5)**. Blood insulin was higher after the dairy treatment and analysis at individual time points showed a significant effect at 30 min (P<0.01) and 60 min (P<0.01). There was an effect of treatment (P<0.0001), time (P<0.01), and a treatment x time interaction (P<0.01) on mean cumulative blood insulin concentrations when expressed as change from baseline. Change from baseline blood insulin scores were significantly higher after the dairy treatment than after the cookie treatment at 30 min (P=0.01) and 60 min (P<0.01). Both mean blood insulin levels and change from baseline blood insulin scores were reduced following the dairy treatment when compared with the cookie treatment (**Figure 5.2**).

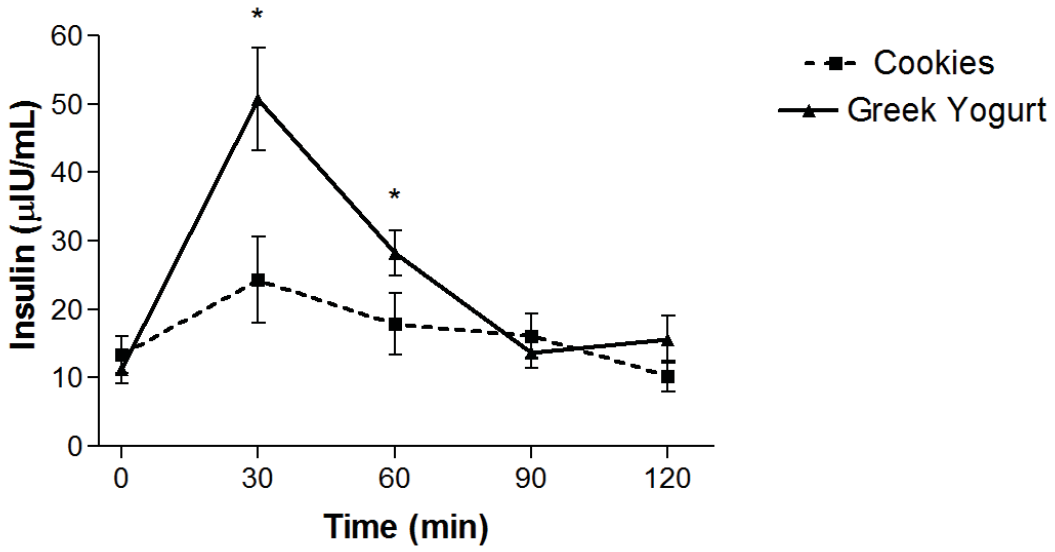
There was an effect of treatment on blood insulin when expressed as tAUC (P<0.01), niAUC (P<0.01) and iAUC (P<0.01). All measures of AUC were greater after the dairy treatment compared to the cookies treatment (**Table 5.5**).

Table 5.5 Blood Insulin Response over 120 minutes

	Treatments	
	Greek Yogurt	Cookies
Mean Blood Insulin (mmol/L)	23.9 ± 3.1 [1,2,3]	16.4 ± 1.9 [1,2,3]
Mean Blood Insulin Change from Baseline (mmol/L)	15.8 ± 3.6 [1,2,3]	3.8 ± 2.0 [1,2,3]
Insulin tAUC (mmoL • min/L)	1857.3 ± 239.3 [1]	654.3 ± 194.7 [1]
Insulin niAUC (mmoL • min/L)	1830.4 ± 242.5 [1]	499.3 ± 244.9 [1]
Insulin iAUC (mmoL • min/L)	3181.8 ± 212.3 [1]	2100.9 ± 378.7 [1]

Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1]an effect of treatment; [2]an effect of time; [3]a time x treatment interaction. P<0.05.

Figure 5.2 Blood Insulin Response over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.
Values with asterisk are significantly different (P<0.05)

5.5 Blood C-Peptide

There was an effect of time (P< 0.01), but not treatment (P=0.16) or a treatment x time interaction (P=0.70) on mean cumulative blood C-peptide concentrations (**Table 5.6**). There was no effect of treatment (P=0.09), time (P=0.16) or a treatment x time interaction (P=0.29) on mean cumulative blood C-peptide concentrations when expressed as change from baseline. Mean blood C-peptide levels and change from baseline levels were not affected by treatment but mean levels increased over time (**Figure 5.3**).

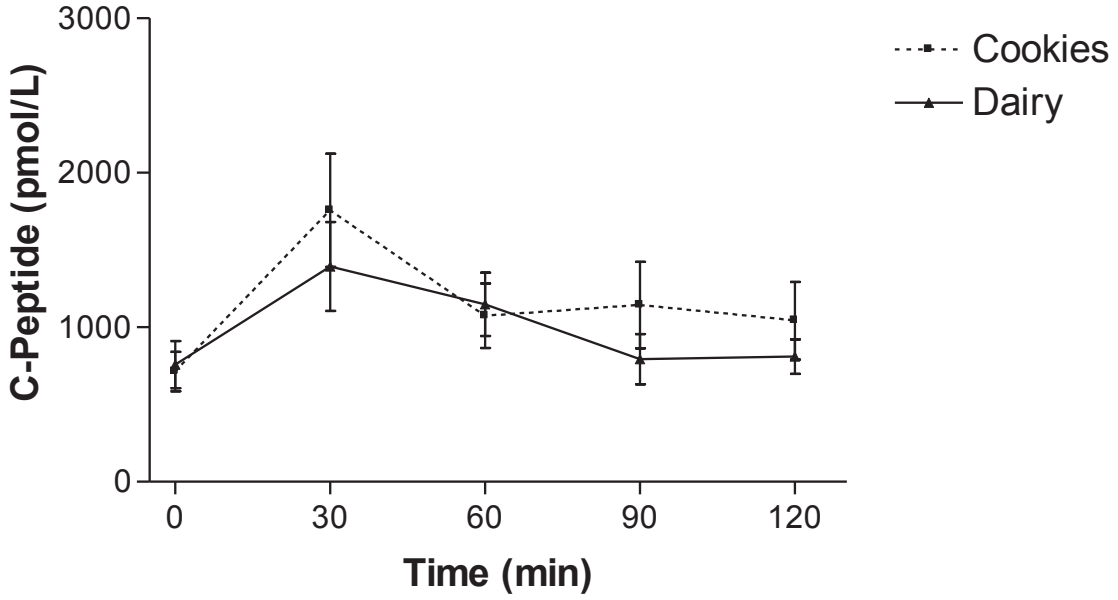
There was no effect of treatment on blood C-peptide tAUC (P=0.31), niAUC (P=0.40), or iAUC (P=0.83) values **(Table 5.6)**.

Table 5.6 Blood C-Peptide Response over 120 minutes

	Treatments	
	Greek Yogurt	Cookies
Mean Blood C-Peptide (pmol/L)	986.0 ± 93.6 [1]	1132.7 ± 122.3 [1]
Mean Blood C-Peptide Change from Baseline (pmol/L)	276.5 ± 84.5	528.8 ± 140.3
C-Peptide tAUC (pmoL • min/L)	33237.7 ± 9557.3	53264.32 ± 1925.33
C-Peptide niAUC (pmoL • min/L)	31457.9 ± 9708.0	49418.4 ± 20405.7
C-Peptide iAUC (pmoL • min/L)	121272.3 ± 23599.0	125541.2 ± 24437.6

Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1]*an effect time. P<0.05.*

Figure 5.3 Blood C-Peptide Response over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.

5.6 Pre-Hepatic Insulin Secretion and Hepatic Insulin Extraction

There was no effect of treatment (P=0.17), time (P=0.52) or a treatment x time interaction (P=0.47) on pre-hepatic insulin secretion as determined by the deconvolution of plasma C-Peptide concentrations (**Figure 5.4**). There was no effect of treatment on insulin secretion tAUC (P=0.11). However, there was a trend that pre-hepatic insulin secretion iAUC was lower after the dairy treatment (P=0.06) and there was an effect of treatment on pre-hepatic insulin secretion niAUC (P=0.05) indicating lower levels after the dairy treatment (**Table 5.7**).

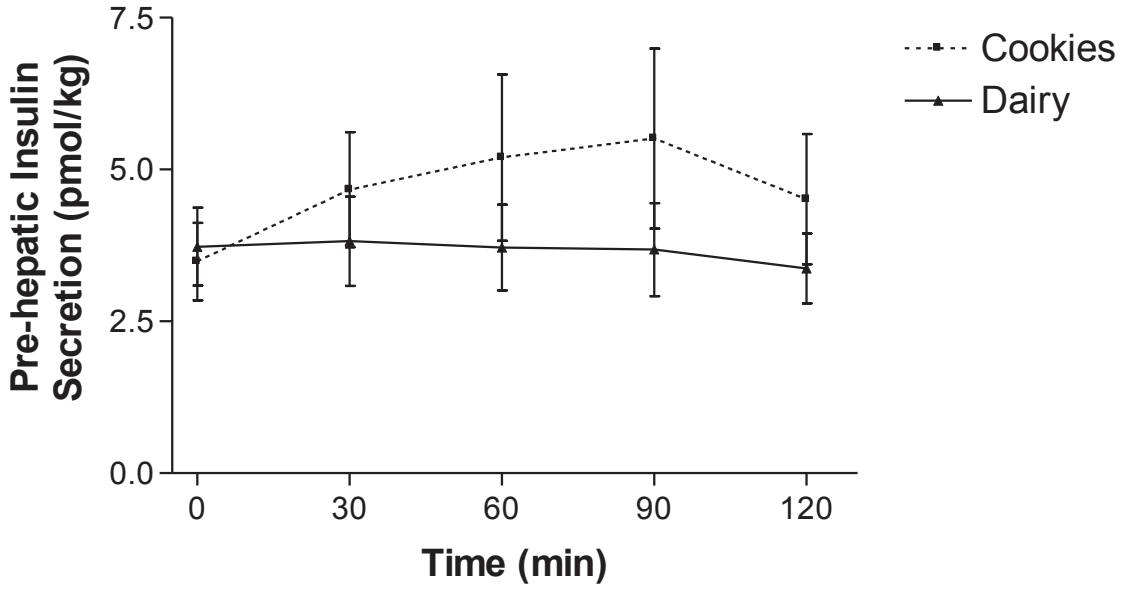
There was an effect of treatment (P=0.001), and a treatment x time interaction (P<0.05) but not time (P=0.49) on hepatic insulin extraction. Overall, hepatic insulin extraction was greater after the cookies treatment compared to the dairy treatment. Analysis of hepatic insulin extraction at the individual time points showed an effect of treatment, indicating that hepatic insulin extraction was lower following the dairy treatment at 30 min (P<0.05) and 60 min (P=0.05) (**Figure 5.5**).

Table 5.7 Mean Pre-Hepatic Insulin Secretion and Hepatic Insulin Extraction

	Treatments	
	Greek Yogurt	Cookies
Pre-Hepatic Insulin Secretion (pmoL/kg)	3.7 ± 0.3	4.7 ± 0.5
Pre-Hepatic Insulin Secretion tAUC (pmoL • min/L)	443.2 ± 82.2	581.3 ± 134.9
Pre-Hepatic Insulin Secretion niAUC (pmoL • min/L)	-4.4 ± 28.4 [1]	163.2 ± 89.2[1]
Pre-Hepatic Insulin Secretion iAUC (pmoL • min/L)	28.0 ± 17.3	181.3 ± 82.0
Hepatic Insulin Extraction	9.2 ± 1.4 [1,3]	14.0 ± 1.8 [1,3]

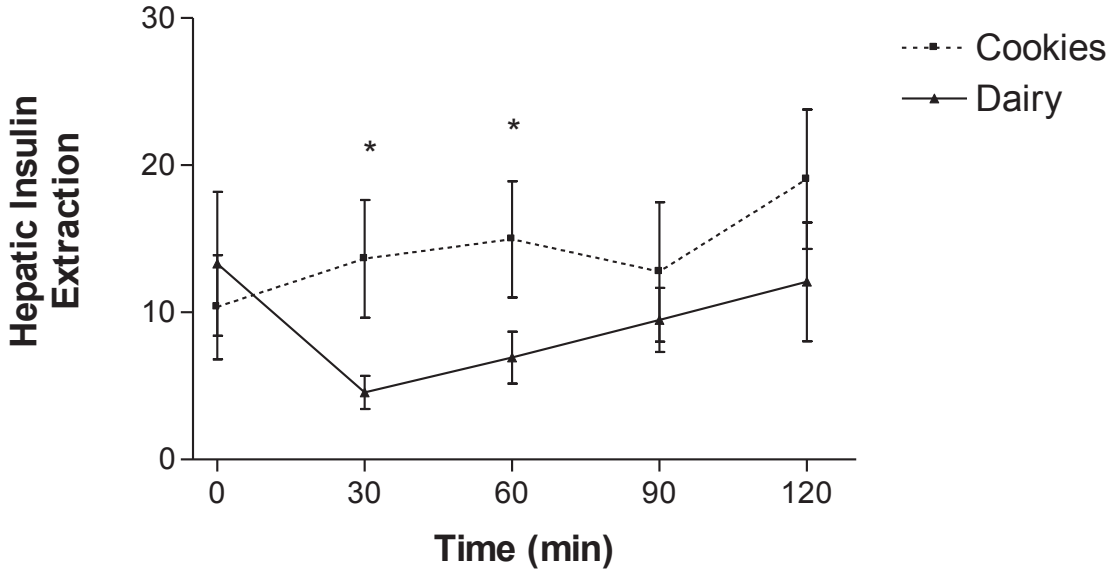
Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1]an effect of treatment; [2]an effect of time; [3]a time x treatment interaction. P<0.05.

Figure 5.4 Pre-Hepatic Insulin Secretion over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.

Figure 5.5 Hepatic Insulin Extraction over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.
Values with asterisk are significantly different (P<0.05).

5.7 Glucose to Insulin Ratios

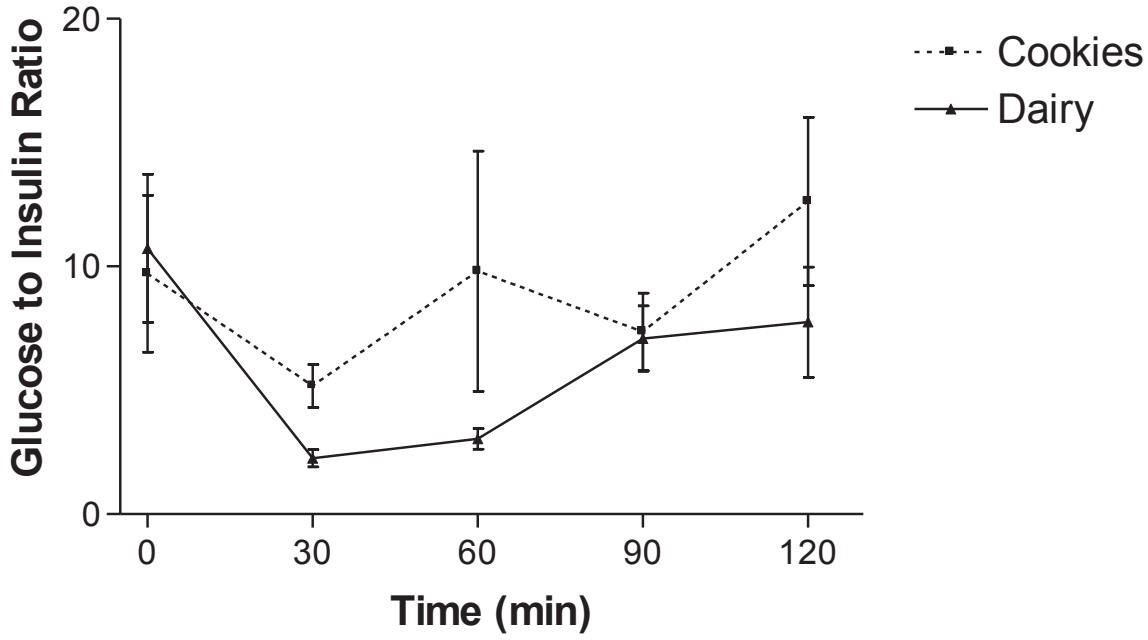
There was an effect of treatment ($P<0.05$) and time ($P=0.01$) but not a treatment x time interaction ($P=0.35$) on glucose to insulin ratios (**Figure 5.6**). Overall, glucose to insulin ratios were higher after the cookies treatment. After the cookies treatment glucose to insulin ratios expressed as iAUC were significantly greater than in the dairy treatment ($P<0.05$) and there was a trend of a similar effect for niAUC ($P=0.07$). However, there was no difference between treatments on glucose to insulin ratios expressed as tAUC ($P=0.21$) (**Table 5.8**).

Table 5.8 Mean Blood Glucose to Insulin Ratios

	Treatments	
	Greek Yogurt	Cookies
Mean Glucose to Insulin Ratio	6.2 ± 0.9 [1,2]	8.9 ± 1.4 [1,2]
Glucose to Insulin Ratio tAUC	0.2 ± 0.1	5.0 ± 3.4
Glucose to Insulin Ratio niAUC	-0.2 ± 0.2	0.5 ± 0.4
Glucose to Insulin Ratio iAUC	3.3 ± 0.3 [1]	6.1 ± 1.1 [1]

Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1]an effect of treatment; [2]an effect of time. P<0.05.

Figure 5.6 Glucose to Insulin Ratios over 120 min



Data are means ± SEM, n = 7.
A one-way ANOVA with a Tukey Kramer post-hoc test.

5.8 Ad libitum Food and Water Intake

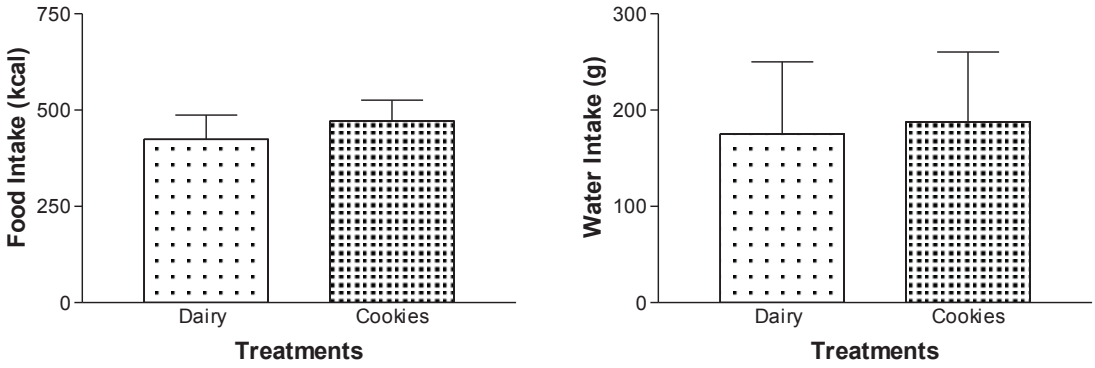
There was no effect of treatment on *ad libitum* food intake (P=0.39), cumulative food intake (treatment + test meal) (P=0.41) or water intake (P=0.51) (**Table 5.9**) (**Figure 5.7**).

Table 5.9 Ad libitum Food and Water Intake

	Treatments	
	Greek Yogurt	Cookies
Food Intake at the Test Meal (kcal)	423.8 ± 63.5	471.8 ± 54.2
Cumulative Food Intake (treatment + test meal intake)	620.3 ± 70.5	667.5 ± 55.8
Water Intake (g)	175.2 ± 74.8	188.0 ± 72.5

Data are means ± SEM, n = 7.
Treatment effects were analyzed using the PROC MEANS with t-test preload treatment as the main factors.

Figure 5.7 Ad libitum Food and Water Intake



Data are means ± SEM, n = 7.
Treatment effects were analyzed using the PROC MEANS with t-test for preload treatments as the main factor.

5.8 Subjective Appetite Scores

5.8.1 Desire to Eat

There was an effect of time (P<0.001) with DTE scores increasing over time however, there was no effect of treatment (P=0.49) or a time x treatment interaction (P=0.93) (**Table 5.10**) (**Figure 5.8**). When expressed as change from baseline there a trend for time (P=0.06) but no effect of treatment (P=0.27) or a time x treatment interaction (P=0.92) (**Figure 5.9**). Total area under the curve (P=0.87), niAUC (P=0.31), iAUC (P=0.33) were no different between treatments for DTE scores (**Table 5.11**).

5.8.2 Hunger

There was an effect of time (P<0.001) with hunger scores increasing over time however, there was no effect of treatment (P=0.46) or a time x treatment interaction (P=0.95) (**Table 5.10**) (**Figure 5.8**). When expressed as change from baseline there was an effect of time (P<0.05), but not treatment (P=0.67) or a time x treatment interaction (P=0.88) (**Figure 5.9**). Total area under the curve (P=0.50), niAUC (P=0.87), iAUC (P=0.63) were no different between treatments for hunger scores (**Table 5.11**).

5.8.3 Fullness

There was no effect of treatment (P=0.18), time (P=0.06) or a time x treatment interaction (P=0.80) on fullness scores (**Table 5.10**) (**Figure 5.8**). When expressed as change from baseline there was an effect of time (P<0.05) and treatment (P=0.05) but not a time x treatment interaction (P=0.81) (**Figure 5.9**). iAUC (P=0.02) but not tAUC (P=0.32) and niAUC (P=0.10) were different between treatments for fullness scores (**Table 5.11**).

5.8.4 Prospective Food Consumption

There was an effect of time (P<0.01), but not treatment (P=0.07) or a time x treatment interaction (P=0.60) on PFC scores (**Table 5.10**) (**Figure 5.8**). When expressed as change from baseline there was an effect of treatment (P=0.01) but not time (P=0.11) or a time x treatment interaction (P=0.82) (**Figure 5.9**). There were no significant differences between treatments for PFC tAUC (P=0.76) and iAUC (P=0.07) but, niAUC (P=0.04) PFC scores were different between treatments (**Table 5.11**).

5.8.5 Average Appetite

There was an effect of time (P<0.01) with AA scores increasing over time however, there was no effect of treatment (P=0.61) or a time x treatment interaction (P=0.66) (**Table 5.10**) (**Figure 5.8**). When AA was expressed as change from baseline there was an effect of treatment (P<0.01) and time (P=0.01) but no treatment x time interaction (P=0.87) (**Figure 5.9**). When expressed as change from the baseline AA scores were lower after the dairy treatment and rose over time. Total area under the curve (P=0.80), niAUC (P=0.19), iAUC (P=0.31) were no different between treatments for AA scores (**Table 5.11**).

5.8.6 Physical Comfort

There was no effect of treatment (P=0.93), time (P=0.07) or their interaction (P=0.96) on absolute physical comfort scores (**Table 5.10**). When expressed as change from baseline there was no effect of treatment (P=0.17), time (P=0.57) or their interaction (P=0.94) on physical comfort scores.

Table 5.10 Absolute Subjective Appetite Scores

	Treatments	
	Greek Yogurt	Cookies
Desire to Eat Absolute (mm)	59.4 ± 3.4 [2]	57.5 ± 3.4 [2]
Desire to Eat Change from Baseline (mm)	13.2 ± 2.4	16.9 ± 3.7
Hunger Absolute (mm)	60.0 ± 3.4 [2]	57.2 ± 3.7 [2]
Hunger Change from Baseline (mm)	21.0 ± 3.5 [2]	18.8 ± 4.7 [2]
Fullness Absolute (mm)	30.2 ± 3.9	35.8 ± 4.4
Fullness Change from Baseline (mm)	-1.5 ± 3.5 [1,2]	-11.4 ± 4.3 [1,2]
Prospective Food Consumption Absolute (mm)	57.5 ± 3.7 [2]	64.6 ± 2.8 [2]
Prospective Food Consumption Change from Baseline (mm)	6.9 ± 2.9 [1]	20.0 ± 3.5 [1]
Average Appetite Absolute (mm)	51.9 ± 3.5 [2]	50.3 ± 3.7 [2]
Average Appetite Change from Baseline (mm)	2.0 ± 3.6 [1,2]	15.2 ± 3.6 [1,2]
Physical Comfort Absolute (mm)	69.2 ± 3.2	69.7 ± 3.3
Physical Comfort Change from Baseline (mm)	5.0 ± 1.9	10.6 ± 3.6

Data are means ± SEM, n = 7.
Two-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent:
[1]an effect of treatment; [2]an effect of time; [3]a time x treatment interaction. P<0.05.

Table 5.11 Area Under the Curve Subjective Appetite Scores

	Treatments	
	Greek Yogurt	Cookies
Average Appetite tAUC	5753.0 ± 1050.2	5982.6 ± 960.7
Average Appetite niAUC	407.1 ± 770.9	1298.3 ± 779.7
Average Appetite iAUC	1075.3 ± 390.2	1651.9 ± 604.0
Desire to Eat tAUC	5844.6 ± 764.8	5923.9 ± 675.6
Desire to Eat niAUC	272.1 ± 458.1	1215.0 ± 827.1
Desire to Eat iAUC	955.5 ± 283.2	1582.3 ± 583.8
Hunger tAUC	6176.8 ± 586.9	5797.5 ± 728.7
Hunger niAUC	1201.1 ± 709.4	1410.0 ± 1058.3
Hunger iAUC	1580.5 ± 495.8	1979.3 ± 589.3
Fullness tAUC	2689.3 ± 784.0	3452.1 ± 1006.46
Fullness niAUC	-573.2 ± 688.0	-1747.5 ± 928.1
Fullness iAUC	398.4 ± 289.0 [1]	324.2 ± 214.2 [1]
Prospective Food Consumption tAUC	6236.8 ± 628.6	6033.2 ± 721.3
Prospective Food Consumption niAUC	-339.6 ± 614.9 [1]	1517.1 ± 614.4 [1]
Prospective Food Consumption iAUC	765.1 ± 301.0	1761.1 ± 430.4

Data are means ± SEM, n = 7. Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve. One-way ANOVA with a Tukey Kramer post-hoc test. Superscripts represent: [1] *an effect of treatment. P<0.05.*

Figure 5.8 Absolute Subjective Appetite Scores

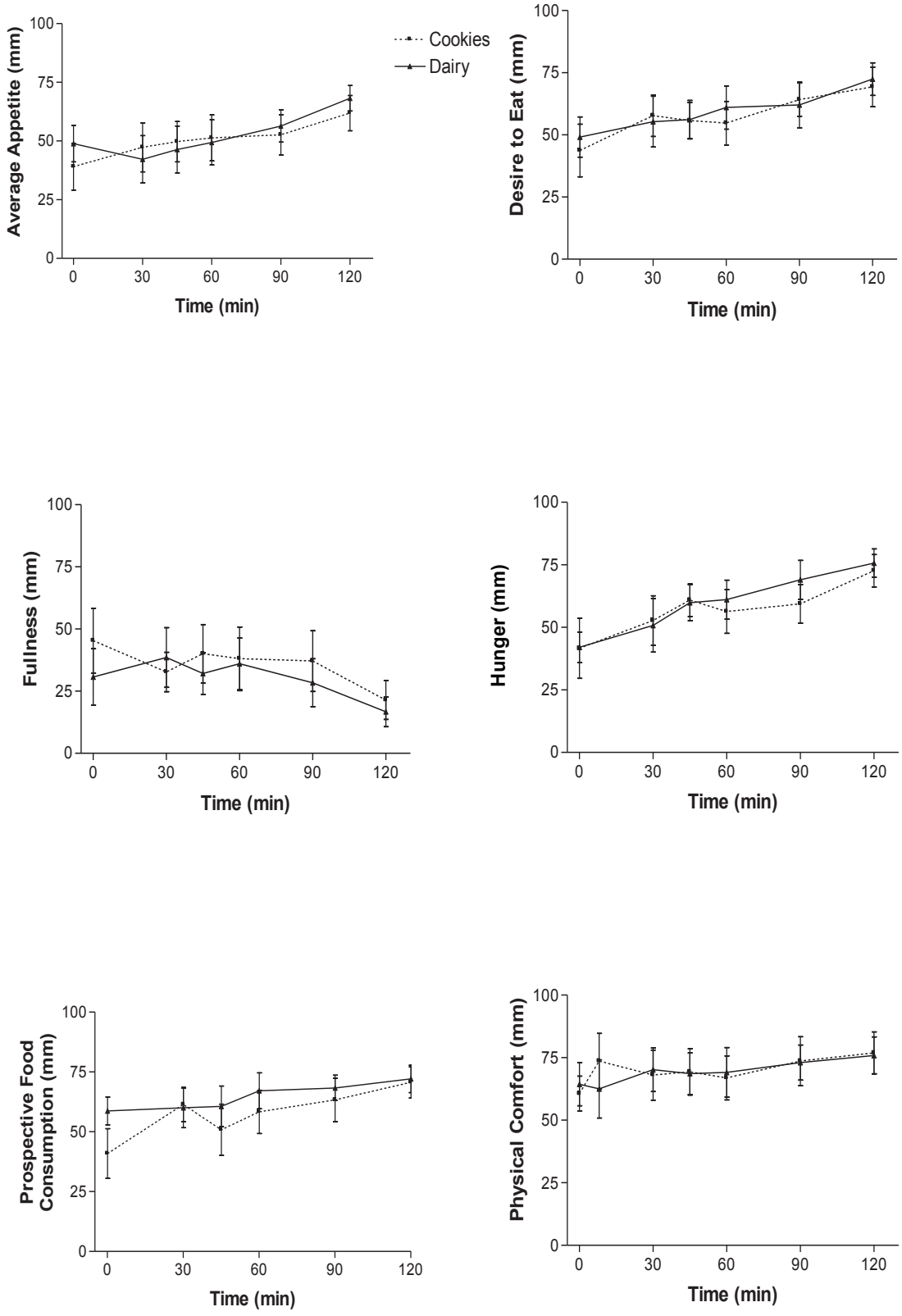
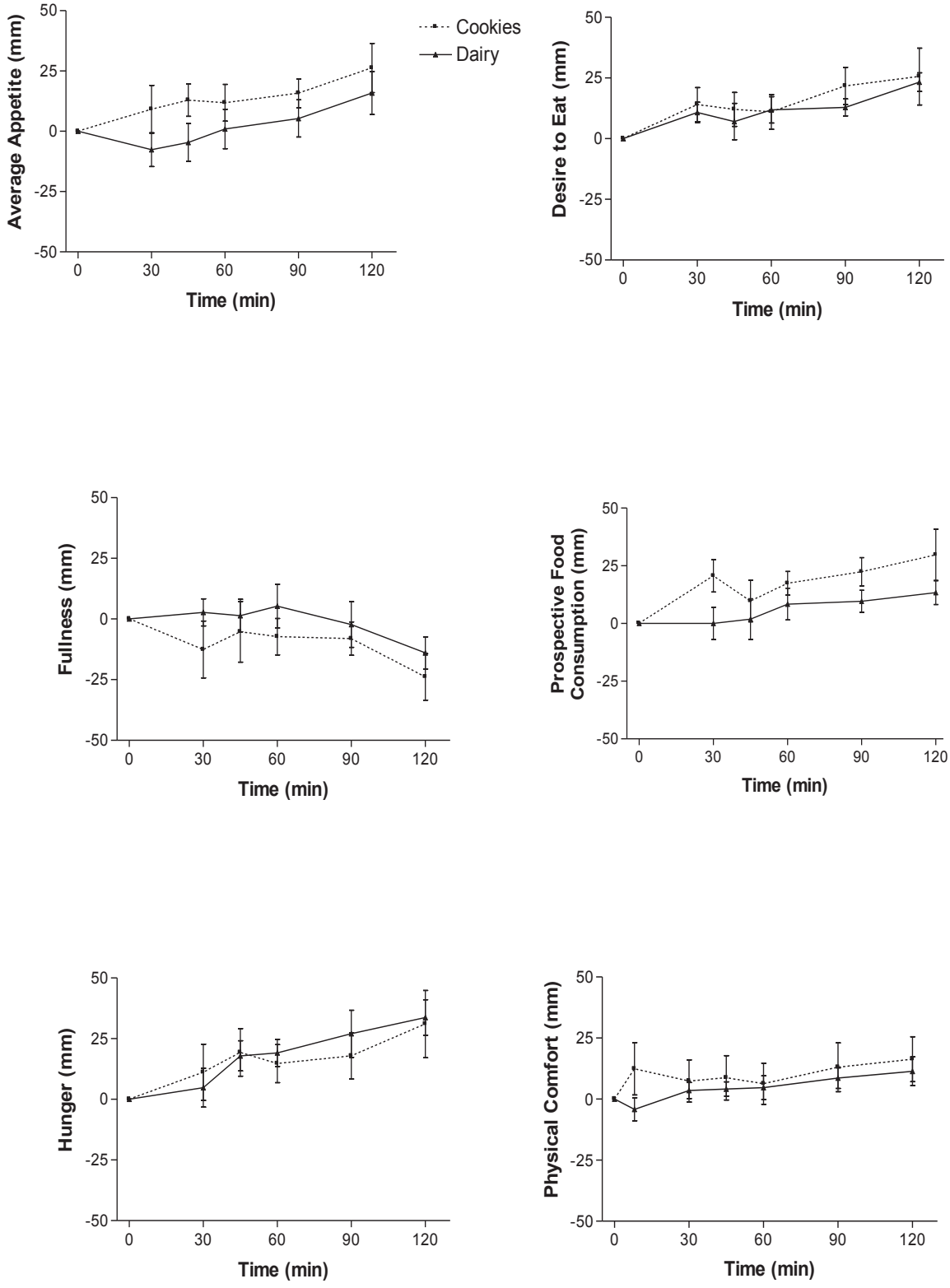


Figure 5.9 Subjective Appetite Change from Baseline Scores



5.9 Relations Among Dependent Measures

5.9.1 Food Intake

Mean BG scores over 120 min were not related to FI (r=0.10192, P=0.73), BG at 120 min was also not related to FI (r=-0.00872, P=0.98). Blood glucose tAUC (r=0.26718, P=0.36), niAUC (r=0.06702, P=0.82) and iAUC (r=0.14228, P=0.63) were not related to FI (**Table 5.12**). Mean insulin scores over 120 min were not related to FI (r=-0.12325, P=0.6746). Insulin at 120 min (r=0.02264, P=0.94) was not related to FI. Blood insulin niAUC (r=-0.52318, P=0.05) and iAUC (r=-0.56821, P<0.05) but not tAUC (r=-0.47553, P=0.09) were related to FI (**Table 5.12**). Mean insulin secretion (ISEC) values over 120 min (r=0.16880, P=0.56) and at 120 min (r=0.24023, P=0.41) were not related to FI. Pre-hepatic insulin secretion tAUC (r=0.37785, P=0.18), niAUC (r=0.36050, P=0.20) and iAUC (r=0.34425, P=0.23) were not related to FI (**Table 5.12**)

Desire to eat scores over 120 min (r=0.36756, P=0.20), at 120 min (r=0.01542, P=0.96), and when expressed as iAUC (r=-0.37481, P=0.19), niAUC (r=-0.36562 P=0.20) or tAUC (r=-0.15226 P=0.60) were not related to FI. Hunger scores over 120 min (r=0.16960, P=0.56) at 120 min (r=0.04591, P=0.88), and when expressed as iAUC (r=0.09531 P=0.75), niAUC (r=0.21483, P=0.46) or tAUC (r=-0.02600, P=0.93) were not related to FI. Fullness scores over 120 min (r=0.33067, P=0.25) at 120 min (r=0.36161, P=0.20), and when expressed as iAUC (r=0.04389, P=0.88), niAUC (r=-0.22755, P=0.43) or tAUC (r=0.27022, P=0.35) were not related to FI. Prospective food consumption scores over 120 min (r=0.16837, P=0.57) at 120 min (r=-0.18823, P=0.52), and when expressed as iAUC (r=-0.21014, P=0.47), niAUC (r=-0.25862, P=0.37) or tAUC (r=-0.19176, P=0.51) were not related to FI. Average Appetite scores over 120 min (r=0.22633, P=0.44) at 120 min (r=0.14563, P=0.62), and when expressed as iAUC (r=0.14312, P=0.63), niAUC (r=0.14671, P=0.62) or tAUC (r=-0.13747 P=0.64) were not related to FI. Physical comfort scores over 120 min (r=0.11036, P=0.01) but not at 120 min (r=-0.11036, P=0.71) were correlated with FI.

The perceived sweetness (r=0.65896, P<0.05) but not pleasantness (r=0.36111, P=0.20) of the treatments was positively correlated with FI.

The emotional eating score from the DEBQ positively correlated with FI ($r=0.53583$, $P<0.05$) but no other measures from the DEBQ were related.

5.9.2 Body Weight

Mean BG scores over 120 min were not related to BW ($r=-0.02847$, $P=0.92$). Blood glucose tAUC ($r=0.55833$, $P<0.05$) but not niAUC ($r=0.42962$, $P=0.13$) and iAUC ($r=-0.19062$, $P=0.51$) was related to BW (**Table 5.13**). Mean insulin scores over 120 min were not related to BW ($r=-0.45090$, $P=0.11$). Blood insulin tAUC ($r=0.42360$, $P=0.13$), niAUC ($r=0.13113$, $=0.66$) and iAUC ($r=0.12904$, $P=0.66$) were related to BW (**Table 5.13**). Mean ISEC values over 120 min ($r=-0.33219$, $P=0.25$) were not related to BW. Insulin secretion values when expressed as tAUC ($r=0.75088$, $P<0.01$) were correlated to BW however, niAUC ($r=0.35557$, $P=0.21$) and iAUC ($r=0.45740$, $P<0.10$) were not (**Table 5.13**). Mean HIE was negatively correlated to BW ($r=-0.55330$, $P<0.05$) but, tAUC ($r=-0.34436$, $P=0.23$), niAUC ($r=-0.34250$, $P=0.23$), and iAUC ($r=-0.37092$, $P=0.19$) were not.

No subjective appetite scores were related to BW (**Table 5.13**).

5.9.3 Blood Glucose

No subjective appetite scores were related to blood glucose (**Table 5.14**).

5.9.4 Blood Insulin

No subjective appetite scores were related to blood insulin (**Table 5.15**).

5.9.5 C-peptide

Mean DTE scores over 120 min ($r=0.56269$, $P<0.05$) and mean fullness scores over 120 min ($r=-0.59394$, $P<0.05$) were correlated to C-peptide levels (**Table 5.16**). No other subjective appetite scores were related to C-peptide levels.

5.9.5 Pre-Hepatic Insulin Secretion

No subjective appetite scores were related to ISEC (**Table 5.17**).

Table 5.12 Correlations of Dependent Measures with Food Intake

Variable	Food Intake
Mean Blood Glucose 0-120 min	r=0.10192
120 min Blood Glucose	r=-0.00872
tAUC Blood Glucose	r=0.26718
niAUC Blood Glucose	r=0.06702
iAUC Blood Glucose	r=0.14228
Mean Insulin 0-120 min	r=-0. 12325
120 min Insulin	r=0.02264
tAUC Insulin	r=-0.47553
niAUC Insulin	r=-0.52318[1]
iAUC Insulin	r=-0.56821[1]
Mean ISEC 0-120 min	r=0.16880
120 min ISEC	r=0.24023
tAUC ISEC	r=0.37785
niAUC ISEC	r=0.36050
iAUC ISEC	r=0.34425
Desire to Eat 0-120 min	r=-0.36756
120 min Desire to Eat	r=0.01542
tAUC Desire to Eat	r=-0.15226
Hunger 0-120 min	r=0.16960
120 min Hunger	r=0.04591
tAUC Hunger	r=-0.02600
Fullness 0-120 min	r=0.33067
120 min Fullness	r=0.36161
tAUC Fullness	r=0.27022
Prospective Food Consumption 0-120 min	r=0.16837
120 min Prospective Food Consumption	r=-018823
tAUC Prospective Food Consumption	r=-0.19176
Average Appetite 0-120 min	r=0.22633
120 min Average Appetite	r=0.14563
tAUC Average Appetite	r=-0.13747
Physical Comfort 0-120 min	r=0.61971[1]
120 min Physical Comfort	r=0.11036
Sweetness	r=0.65896[1]
Pleasantness	r=0.36111
Tanner Stage	r=0.47323
DEBQ Emotional Eating Score	r=0.53583[1]
DEBQ Average	r=0.47008

Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve.
Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Table 5.13 Correlations of Dependent Measures with Body Weight

Variable	Body Weight
Mean Blood Glucose 0-120 min	r=-0.02847
tAUC Blood Glucose	r=0.55833[1]
niAUC Blood Glucose	r=0.42962
iAUC Blood Glucose	r=-0.19062
Mean Insulin 0-120 min	r=-0.45090
tAUC Insulin	r=0.42360
niAUC Insulin	r=0.13113
iAUC Insulin	r=0.12904
Mean ISEC 0-120 min	r=0.16880
tAUC ISEC	r=0.75088[1]
niAUC ISEC	r=0.35557
iAUC ISEC	r=0.45740
Mean HIE 0-120 min	r=-0.55330[1]
tAUC HIE	r=-0.34426
niAUC HIE	r=-0.34250
iAUC HIE	r=-0.37092
Mean GI Ratio 0-120 min	r=-0.24091
tAUC GI Ratio	r=-0.42100
niAUC GI Ratio	r=0.40030
iAUC GI Ratio	r=-0.30678
Desire to Eat 0-120 min	r=-0.41571
tAUC Desire to Eat	r=-0.10061
Hunger 0-120 min	r=-0.00202
tAUC Hunger	r=-0.13570
Fullness 0-120 min	r=0.50742
tAUC Fullness	r=-0.20978
Prospective Food Consumption 0-120 min	r=0.00966
tAUC Prospective Food Consumption	r=-0.06702
Average Appetite 0-120 min	r=-0.06231
tAUC Average Appetite	r=-0.22652

Abbreviations: tAUC, total area under the curve, niAUC, net incremental area under the curve; iAUC, incremental area under the curve; ISEC, insulin secretion; HIE, hepatic insulin extraction; GI, glucose to insulin.
Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Table 5.14 Correlations of Dependent Measures with Blood Glucose

Variable	Glucose
Desire to Eat 0-120 min	r=0.01568
120 min Desire to Eat	r=-0.42150
Hunger 0-120 min	r=-0.39623
120 min Hunger	r=-0.23342
Fullness 0-120 min	r=-0.11495
120 min Fullness	r=0.08700
Prospective Food Consumption 0-120 min	r=-0.15379
120 min Prospective Food Consumption	r=-0.27383
Average Appetite 0-120 min	r=-0.40595
120 min Average Appetite	r=-0.29213

Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Table 5.15 Correlations of Dependent Measures with Blood Insulin

Variable	Insulin
Desire to Eat 0-120 min	r=0.17036
120 min Desire to Eat	r=0.04815
Hunger 0-120 min	r=0.01803
120 min Hunger	r=0.03613
Fullness 0-120 min	r=-0.41688
120 min Fullness	r=-0.06462
Prospective Food Consumption 0-120 min	r=0.14488
120 min Prospective Food Consumption	r=-0.04320
Average Appetite 0-120 min	r=-0.23126
120 min Average Appetite	r=0.36050

Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Table 5.16 Correlations of Dependent Measures with Blood C-peptide

Variable	C-Peptide
Desire to Eat 0-120 min	r=0.56269[1]
120 min Desire to Eat	r=-0.27154
Hunger 0-120 min	r=0.35377
120 min Hunger	r=-0.45621
Fullness 0-120 min	r=-0.59394[1]
120 min Fullness	r=0.23909
Prospective Food Consumption 0-120 min	r=0.47192
120 min Prospective Food Consumption	r=-0.47984
Average Appetite 0-120 min	r=-0.15535
120 min Average Appetite	r=-0.32297

Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Table 5.17 Correlations of Dependent Measures with Pre-hepatic Insulin Secretion

Variable	C-Peptide
Desire to Eat 0-120 min	r=0.49785
120 min Desire to Eat	r=-0.32941
Hunger 0-120 min	r=0.34677
120 min Hunger	r=-0.48004
Fullness 0-120 min	r=0.49413
120 min Fullness	r=0.38965
Prospective Food Consumption 0-120 min	r=-0.40093
120 min Prospective Food Consumption	r=0.34039
Average Appetite 0-120 min	r=-0.29005
120 min Average Appetite	r=-0.23139

Pearson Correlation Coefficients. [1]Indicates Significance (P<0.05).

Chapter 6: Discussion

The observed increase in circulating insulin, reduction in blood glucose and no difference in C-peptide after a dairy snack compared to a non-dairy snack is evidence that available carbohydrate alone does not predetermine the glycemic response of a snack. The improvement in post-prandial glycemic control after the dairy treatment supports the original hypothesis however, how this control is achieved through a different mechanism than proposed (increased insulin secretion).

Mean blood insulin (**Figure 5.1**) was greater after the dairy treatment at 30 and 60 min and mean blood glucose over 120 min (**Figure 5.2**) was reduced however, the reduction in blood glucose was not attributable to any given time point and this is potentially due to the small number of participants in the study. For example, in a study of identical design using 11 normal weight children similar results were found for insulin but blood glucose was significantly reduced at 60 and 90 min (unpublished results). It is also possible that because over weight and obese children have higher blood glucose levels (143) changes in blood glucose that may be detectable in normal weight children may not be large enough to be detected by over weight and obese children.

Another potential explanation is the unique effect of milk products to increase insulinaemia to a greater degree than can be explained by the concurrent glycemia alone; this has been observed previously in adults (92, 144). The exact protein composition of the Greek yogurt used in this study is unknown however, typical Greek yogurt composition suggests it is primarily casein (145). Regardless of the exact protein ratio of the treatment used in the study it is known that both whey protein (146) and casein protein (147) possess insulinotropic properties. It has been shown that there is a strong correlation between the postprandial insulin response and the rate at which leucine, valine, lysine and isoleucine appear in the blood stream (148). Leucine, valine, lysine and isoleucine are all amino acids found in high quantities in milk protein particularly the whey fraction (148). Furthermore, milk proteins have been shown to stimulate incretin release in humans (149) and decrease the activity of dipeptidyl peptidase IV (DPP-IV) which deactivates GLP-1 as it was shown in mice (150). Therefore, it is likely that the protein in the dairy product, particularly the branched chain amino acids, could have caused such high levels of circulating insulin. However, C-peptide (**Figure 5.3**) and pre-

hepatic insulin secretion (**Figure 5.4)** were not different between treatments indicating the observed increase in circulating insulin was not due to a greater production of insulin which may have induced additional stress on β-cells (151).

Two possible mechanisms for the disparity between circulating insulin and C-peptide levels are increased hepatic insulin clearance and increased insulin efficiency. Hepatic insulin extraction was reduced after the dairy treatment at 30 and 60 min, the same time points as insulin levels were increased. A study in over weight adolescents demonstrated that chronic supplementation of casein or whey independently, but not skim milk for 12 weeks increased fasting C-peptide levels suggesting a unique effect of whole milk foods, similar to the dairy product in this study, on insulin secretion (152). In the short term, previous work by Lan-Pidhainy and Wolever in healthy adults found that whey protein when consumed with fat and carbohydrate resulted in a greater insulin response independent of C-peptide and ISEC (149) than fat and carbohydrate alone and is in agreement with the current results. Blood C-peptide divided by circulating insulin provides a measure of the clearance of insulin when travelling from the islets of Langerhans to the portal vein and is estimated to be about 50% before entering circulation when fasted (153). It is estimated that feeding reduces hepatic insulin clearance by 20% (73) but there is limited data available about the effect of specific nutrients to attenuate this clearance. Lan-Pidhainy and Wolever postulated that the amino acids that contribute the insulinemic effect of whey protein act as competitive inhibitors by binding to insulin receptors on the hepatocytes thereby reducing the amount of hepatic clearance (149). Administration of GLP-1 in mice led to a reduction in HIE (154) as did infusion of GIP in young relatives of Type II diabetics (155). This mechanism is plausible as milk protein not only stimulates incretin release but also inhibits DPP-IV action. However, it was later shown that HIE was not affected by endogenously produced circulating levels of GLP-1 and GIP in humans in addition an inverse relationship between insulin plasma concentrations and insulin clearance rates was found (156). These results indicate that insulin production itself controls HIE and while the mechanism is unknown the authors suggest it may be through saturation of receptor-mediated endocytosis. If insulin secretion is the mediator of HIE it would have been expected that there was a difference in ISEC that was not observed. Additionally, it

was also found that ingestion of glucose compared to infusion of glucose reduced the rate of HIE implicating factors associated with digestion and absorption of nutrients in the stomach and small intestine in the regulation of HIE (156). An alternative rationale for the observed difference in glycemia is the increased effectiveness of insulin.

Blood glucose was affected by time and treatment similarly to the glucose to insulin (G:I) ratio. The G:I ratio has been used previously to determine the efficiency of insulin action between different people (148, 149) but has also recently been used to deteremine differences within the same individuals after ingestion of nutrients (157). Using this method it can be postulated that the high circulating levels of insulin was due to increased efficacy over 120 min in the dairy treatment compared to the cookies treatment.

It is also possible that the reduction in glycemia after the dairy treatment compared to the non-dairy treatment may have not been a result of dairy proteins and their effects on insulin action but on the type of carbohydrate in each treatment itself. The total number of available carbohydrates was identical between treatments however, the presence of lactose in the dairy treatment would not have been present in the cookies treatment. The analysis of a similar product (President Choice Strawberry Yogurt, Loblaws Inc., Canada) found that 12% (3 g) of the total carbohydrate content was lactose with the remaining being a combination of sucrose, glucose and fructose. The effect of lactose is likely to not have contributed to the insulinemic and glycemic response to the same degree as the dairy proteins as lactose in isolation (24 g) has been shown to have a far smaller effect on insulin in comparison to fermented milk products (92).

Contrary to the original hypothesis FI at the test meal did not differ between treatments. It is possible this was due to the weight status of the participants (over weight or obese), small caloric dose of the treatment (170.5-175 kcal), small sample size (7 participants), long time to the test meal (120 min) and that the participants were not truly fasted as they had consumed a standardized breakfast. Previous research has shown that a group of 17 9-14 year old over weight and obese boys were able to compensate for 25 g (200 kcal) of glucose given 30 min before an *ad libitum* test meal however, their response to an isocaloric load of whey protein was impaired compared to their normal weight counterparts (10). Therefore, it is unexpected that a smaller group of over weight and

obese boys would be expected to compensate to a greater degree to a smaller preload given a longer time before a test meal. It is likely that the time to next meal was a greater determinant in the lack of effect of FI at the subsequent meal then the size or composition of the treatments as there was no correlation between the energy (r=-0.18121, P=0.54), protein (r=-0.21491, P=0.46), or carbohydrate (r=-0.19053, P=0.51) per kg of BW provided by the preloads to subsequent FI. This finding was similar to another study comparing dairy beverages to other commercially available beverages, where differences in FI were seen at 60 min but not at 120 min test meal (158). Furthermore, no difference in *ad libitum* FI was observed after 20 adult men consumed varying dairy snacks or orange juice with calorie content ranging from 141 to 167 kcal two hours later despite the different types of foods provided and their protein to carbohydrate ratios (159). Similarly, in adult women no differences in meal initiation or subsequent FI was observed when 160 kcal yogurt snacks were given in the afternoon (160). While higher levels of circulating insulin levels and reduced levels of glucose have been associated with reduced FI (161) differences in insulin and glucose in this study were not shown to have an effect on FI. However, this is likely due to the fact that differences in insulin levels were no longer present after 60 min and any anorexigenic action of insulin may have exerted would have dissipated.

All subjective appetite measures rose over time and dropped significantly after the test meal supporting other studies that have shown the vailidity of using VAS to determine subjective appetite in children (10, 128, 133, 162). When subjective appetite scores were expressed as change from baseline AA was higher after the cookies treatment and fullness lower. However, because there was no difference in FI it was expected these measures of subjective appetite would not correlate with FI and they did not. At no time point did blood glucose or insulin levels correlate with subjective appetite measures suggesting that any anorexigenic properties of insulin cannot explain changes in subjective appetite. Although, average DTE and fullness scores correlated with C-peptide levels implying that insulin production rather than circulating levels *per se* may affect subjective appetite but, with the limited sample size it is difficult to infer if an actual relationship exists.

The strongest VAS predictor of FI was PC but PC was not different between treatments. The relation of FI to PC may be due to inter-participant differences in comfort with the blood drawing techniques implying that children who are more comfortable with invasive procedures are more likely not to have their appetite affected. Paradoxically, children who scored higher on the emotional eating section of the DEBQ, meaning they are more likely to eat in response to emotions (ie. negative emotions associated with invasive procedures) were associated with higher FI during the study (163). It is possible that PC alone was unable to capture all of the emotions associated with all aspects of the study in a way conducive to predicting eating behavior.

A potential limitation of the study was the length between the treatment and the next meal (120 min) as likelihood of identifying an effect on FI was greatly diminished. The intention of this study was to replicate normal eating behaviours of young children therefore, two hours after breakfast and before lunch represents a morning snack. A lack of effect on FI is an important finding and an example of the experiment conditions matching real world conditions rather than those most conducive to a favourable result.

The strength of this study was the homogeneity of the sample and that treatments were foods normally consumed by children and provided in typically consumed amounts.

Chapter 7: Practical Application

The current study has provided support for young children to consume dairy products, Greek yogurt in particular, due to their favourable impact on glycemic regulation. This research is timely, as the number of high-protein dairy snacks including Greek yogurt products commercially available has increased. Establishing a dietary behavior that positively influences glycemic control while also providing a number of micronutrients early in life may contribute to better metabolic health throughout the lifespan. The use of a commercially available product that combines macronutrients provides a result that is easily translatable to health professionals and the lay public alike. Consuming dairy products in conjunction with higher glycemic foods may be able to reduce postprandial glycemia without an over production of insulin.

Chapter 8: Conclusion

In 9-14 y old over weight and obese boys a dairy snack reduced glycemia and increased circulating insulin levels by a reduction in hepatic insulin secretion 120 min after consumption compared to a non-dairy snack matched for available carbohydrate without affecting subjective appetite and subsequent food intake. Dairy proteins are likely to have unique and favourable insulinotropic effects.

References

1. Shields M. Overweight and obesity among children and youth. Health reports / Statistics Canada, Canadian Centre for Health Information. 2006;17(3):27-42.
2. Dixon JB. The effect of obesity on health outcomes. Mole Cell Endocrinol 2010;316(2):104-8.
3. Aanstoot HJ, Anderson BJ, Daneman D, et al. The global burden of youth diabetes: perspectives and potential. Pediatr diabetes 2007;8 Suppl 8:1-44.
4. Amed S, Dean HJ, Panagiotopoulos C, et al. Type 2 diabetes, medication-induced diabetes, and monogenic diabetes in Canadian children: a prospective national surveillance study. Diabetes Care 2010;33(4):786-91.
5. Wadsworth LA, McHugh TL, Thompson AM, et al. Dietary intake of Nova Scotia youth in grades 7 and 11. Can J Diet Pract Res 2012;73(1):14-20.
6. Akhavan T, Luhovyy BL, Anderson GH. Effect of drinking compared with eating sugars or whey protein on short-term appetite and food intake. Int J Obes 2011;35(4):562-9.
7. Akhavan T, Luhovyy BL, Brown PH, Cho CE, Anderson GH. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. Am J Clin Nutr 2010;91(4):966-75.
8. Luhovyy BL, Akhavan T, Anderson GH. Whey proteins in the regulation of food intake and satiety. J Am Coll Nutr 2007;26(6):704S-12S.
9. Major GC, Chaput JP, Ledoux M, et al. Recent developments in calcium-related obesity research. Obes Rev 2008;9(5):428-45.
10. Bellissimo N, Desantadina MV, Pencharz PB, Berall GB, Thomas SG, Anderson GH. A comparison of short-term appetite and energy intakes in normal weight and obese boys following glucose and whey-protein drinks. Int J Obes (Lond) 2008;32(2):362-71.
11. Patel BP, Bellissimo N, Luhovyy B, et al. An after-school snack of raisins lowers cumulative food intake in young children. J Food Sci 2013;78 Suppl 1:A5-A10.
12. Patel BP, Luhovyy B, Mollard R, Painter JE, Anderson GH. A premeal snack of raisins decreases mealtime food intake more than grapes in young children. Appl Physiol Nutr Metab 2013;38(4):382-9.
13. Blaak EE, Antoine JM, Benton D, et al. Impact of postprandial glycaemia on health and prevention of disease. Obes Rev 2012;13(10):923-84.
14. Goldenberg R, Punthakee Z. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. Can J Diabetes 2013;37, Supplement 1(0):S8-S11.
15. Uysal Y, Wolters B, Knop C, Reinehr T. Components of the metabolic syndrome are negative predictors of weight loss in obese children with lifestyle intervention. Clin Nutr 2013.
16. Roberts KC, Shields M, de Groh M, Aziz A, Gilbert JA. Overweight and obesity in children and adolescents: results from the 2009 to 2011 Canadian Health Measures Survey. Health Rep 2012;23(3):37-41.
17. Shields M. Overweight and obesity among children and youth. Health Rep 2006;17(3):27-42.

18. Shields M, Tremblay MS. Canadian childhood obesity estimates based on WHO, IOTF and CDC cut-points. Pediatr Obes 2010;5(3):265-73.
19. Public Health Agency of Canada. Obesity in Canada: A joint report from the Public Health Agency of Canada and the Canadian Institute for Health Information. 2011.
20. Kirk SF, Kuhle S, Ohinmaa A, Veugelers PJ. Health behaviours and health-care utilization in Canadian schoolchildren. Public Health Nutr 2013;16(2):358-64.
21. Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev 2008;9(5):474-88.
22. Dores H, de Araujo Goncalves P, Carvalho MS, et al. Body mass index as a predictor of the presence but not the severity of coronary artery disease evaluated by cardiac computed tomography. Eur J Prev Caridol 2013.
23. Nguyen NT, Magno CP, Lane KT, Hinojosa MW, Lane JS. Association of hypertension, diabetes, dyslipidemia, and metabolic syndrome with obesity: findings from the National Health and Nutrition Examination Survey, 1999 to 2004. J Am Coll Surg 2008;207(6):928-34.
24. Association CD. 2013 Clinical Practice Guidelines. Can J Diabetes 2013;37:S1-212.
25. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358(9277):221-9.
26. American Diabetes Association. Standards of medical care in diabetes--2013. Diabetes Care 2013;36 Suppl 1:S11-66.
27. Danaei G, Finucane MM, Lu Y, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 2011;378(9785):31-40.
28. Pelletier C, Dai S, Roberts KC, Bienek A, Onysko J, Pelletier L. Report summary. Diabetes in Canada: facts and figures from a public health perspective. Chronic Dis Inj Can 2012;33(1):53-4.
29. Demmer RT, Zuk AM, Rosenbaum M, Desvarieux M. Prevalence of Diagnosed and Undiagnosed Type 2 Diabetes Mellitus Among US Adolescents: Results From the Continuous NHANES, 1999-2010. Am J Epidemiol 2013;178(7):1106-13.
30. Group TS, Zeitler P, Hirst K, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. N Engl J Med 2012;366(24):2247-56.
31. Berends LM, Ozanne SE. Early determinants of type-2 diabetes. Best Pract Res Clin Endocrinol Metab 2012;26(5):569-80.
32. Song SH, Hardisty CA. Early-onset Type 2 diabetes mellitus: an increasing phenomenon of elevated cardiovascular risk. Exper Rev Cardiovasc Ther 2008;6(3):315-22.
33. Song SH, Hardisty CA. Early onset type 2 diabetes mellitus: a harbinger for complications in later years--clinical observation from a secondary care cohort. QJM 2009;102(11):799-806.
34. Grundy SM, Brewer HB, Jr., Cleeman JI, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart

Association conference on scientific issues related to definition. Circulation 2004;109(3):433-8.
35. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120(16):1640-5.
36. Statistics Canada. Metabolic syndrome in Canadians, 2009 to 2011. Health Facts, 2013.
37. Steinberger J, Daniels SR, Eckel RH, et al. Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. Circulation 2009;119(4):628-47.
38. Lawlor DA, Benfield L, Logue J, et al. Association between general and central adiposity in childhood, and change in these, with cardiovascular risk factors in adolescence: prospective cohort study. BMJ 2010;341.
39. St-Onge MP, Keller KL, Heymsfield SB. Changes in childhood food consumption patterns: a cause for concern in light of increasing body weights. Am J Clin Nutr 2003;78(6):1068-73.
40. Smith LP, Ng SW, Popkin BM. Trends in US home food preparation and consumption: analysis of national nutrition surveys and time use studies from 1965-1966 to 2007-2008. Nutr J 2013;12:45.
41. Jahns L, Siega-Riz AM, Popkin BM. The increasing prevalence of snacking among US children from 1977 to 1996. J Pediatr 2001;138(4):493-8.
42. Poti JM, Popkin BM. Trends in energy intake among US children by eating location and food source, 1977-2006. J Am Diet Assoc 2011;111(8):1156-64.
43. Gilbert JA, Miller D, Olson S, St-Pierre S. After-school snack intake among Canadian children and adolescents. Can J Public Health 2012;103(6):e448-52.
44. Farley TA, Baker ET, Futrell L, Rice JC. The ubiquity of energy-dense snack foods: a national multicity study. Am J Public Health 2010;100(2):306-11.
45. Lien N, Lytle LA, Klepp KI. Stability in consumption of fruit, vegetables, and sugary foods in a cohort from age 14 to age 21. Prev Med 2001;33(3):217-26.
46. Larson NI, Neumark-Sztainer D, Hannan PJ, Story M. Trends in adolescent fruit and vegetable consumption, 1999-2004: project EAT. Am J Prev Med 2007;32(2):147-50.
47. Demory-Luce D, Morales M, Nicklas T, Baranowski T, Zakeri I, Berenson G. Changes in food group consumption patterns from childhood to young adulthood: the Bogalusa Heart Study. J Am Diet Assoc 2004;104(11):1684-91.
48. Driskell JA, Kim YN, Goebel KJ. Few differences found in the typical eating and physical activity habits of lower-level and upper-level university students. J Am Diet Assoc 2005;105(5):798-801.
49. Drummond S, Crombie N, Kirk T. A critique of the effects of snacking on body weight status. Eur J Clin Nutr 1996;50(12):779-83.

50. Bellisle F, Dalix AM, Mennen L, et al. Contribution of snacks and meals in the diet of French adults: a diet-diary study. Physiol Behav 2003;79(2):183-9.
51. Popkin BM, Duffey KJ. Does hunger and satiety drive eating anymore? Increasing eating occasions and decreasing time between eating occasions in the United States. Am J Clin Nutr 2010;91(5):1342-7.
52. Hampl JS, Heaton CL, Taylor CA. Snacking patterns influence energy and nutrient intakes but not body mass index. J Hum Nutr Diet 2003;16(1):3-11.
53. Sebastian RS, Cleveland LE, Goldman JD. Effect of snacking frequency on adolescents' dietary intakes and meeting national recommendations. J Adolesc Health 2008;42(5):503-11.
54. Masse LC, Blanck HM, Valente M, et al. Association between self-reported household practices and body mass index of US children and adolescents, 2005. Prev Chronic Dis 2012;9:E174.
55. Peneau S, Thibault H, Meless D, et al. Anthropometric and behavioral patterns associated with weight maintenance after an obesity treatment in adolescents. J Pediatr 2008;152(5):678-84.
56. Jennings A, Cassidy A, van Sluijs EM, Griffin SJ, Welch AA. Associations between eating frequency, adiposity, diet, and activity in 9-10 year old healthy-weight and centrally obese children. Obesity 2012;20(7):1462-8.
57. Tan SY, Mattes RD. Appetitive, dietary and health effects of almonds consumed with meals or as snacks: a randomized, controlled trial. Eur J Clin Nutr 2013;67(11):1205-14.
58. United States Department of Agriculture. Fluid milk consumption in the United States: What we eat in America, NHANES 2005-2006. Food Surveys Research Dietary Data Brief. Beltsville Human Nutrition Research Center, 2012.
59. Bradlee ML, Singer MR, Qureshi MM, Moore LL. Food group intake and central obesity among children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES III). Public Health Nutr 2010;13(6):797-805.
60. Moore LL, Bradlee ML, Gao D, Singer MR. Low dairy intake in early childhood predicts excess body fat gain. Obesity 2006;14(6):1010-8.
61. Kelishadi R, Zemel MB, Hashemipour M, Hosseini M, Mohammadifard N, Poursafa P. Can a dairy-rich diet be effective in long-term weight control of young children? J Am Coll Nutr 2009;28(5):601-10.
62. Sokoloff L, Reivich M, Kennedy C, et al. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 1977;28(5):897-916.
63. Edvinsson L ME, McCulloch J. Cerebral blood flow and metabolism. New York: Raven Press, 1993.
64. Mathers J WT. Introduction to Human Nutrition, 2009.
65. Medicine Io. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids (macronutrients). Washington: National Academies Press, 2005.
66. Just T, Pau HW, Engel U, Hummel T. Cephalic phase insulin release in healthy humans after taste stimulation? Appetite 2008;51(3):622-7.

67. Hutton JC. Insulin secretory granule biogenesis and the proinsulin-processing endopeptidases. Diabetologia 1994;37 Suppl 2:S48-56.
68. Horwitz DL, Rubenstein AH, Reynolds C, Molnar GD, Yanaihara N. Prolonged suppression of insulin release by insulin-induced hypoglycemia: demonstration by C-peptide assay. Horm Metab Res 1975;7(6):449-52.
69. Lavin JH, Wittert GA, Andrews J, et al. Interaction of insulin, glucagon-like peptide 1, gastric inhibitory polypeptide, and appetite in response to intraduodenal carbohydrate. Am J Clin Nutr 1998;68(3):591-8.
70. De Vos A, Heimberg H, Quartier E, et al. Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J Clin Invest 1995;96(5):2489-95.
71. Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. Endocr Rev 1998;19(5):608-24.
72. Zavaroni I, Deferrari G, Lugari R, et al. Renal metabolism of C-peptide in man. J Clin Endocrinol Metab 1987;65(3):494-8.
73. Caumo A, Florea I, Luzi L. Effect of a variable hepatic insulin clearance on the postprandial insulin profile: insights from a model simulation study. Acta Diabetol 2007;44(1):23-9..
74. Polonsky KS, Given BD, Hirsch L, et al. Quantitative study of insulin secretion and clearance in normal and obese subjects. J Clin Invest 1988;81(2):435-41.
75. Marini MA, Frontoni S, Succurro E, et al. Differences in insulin clearance between metabolically healthy and unhealthy obese subjects. Acta Diabetol 2014;51(2):257-61.
76. Mojsov S, Kopczynski MG, Habener JF. Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. J Biol Chem 1990;265(14):8001-8.
77. Ferrannini E. Physiology of glucose homeostasis and insulin therapy in type 1 and type 2 diabetes. Endocrinol Metab Clin North Am 2012;41(1):25-39.
78. Elrick H, Stimmler L, Hlad CJ, Jr., Arai Y. Plasma Insulin Response to Oral and Intravenous Glucose Administration. J Clin Endocrinol Metab 1964;24:1076-82.
79. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 1973;37(5):826-8.
80. Burcelin R, Da Costa A, Drucker D, Thorens B. Glucose competence of the hepatoportal vein sensor requires the presence of an activated glucagon-like peptide-1 receptor. Diabetes 2001;50(8):1720-8.
81. Schirra J, Katschinski M, Weidmann C, et al. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97(1):92-103.
82. Natali A, Toschi E, Camastra S, Gastaldelli A, Groop L, Ferrannini E. Determinants of postabsorptive endogenous glucose output in non-diabetic subjects. European Group for the Study of Insulin Resistance (EGIR). Diabetologia 2000;43(10):1266-72.
83. Mayer J. The glucostatic theory of regulation of food intake and the problem of obesity. Bull New Engl Med 1952;14(2):43.
84. Mayer J. Glucostatic mechanism of regulation of food intake. New Engl J Med 1953;249(1):13.

85. Mayer J. Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Ann N Y Acad Sci 1955;63(1):15-43.
86. Smith GP, Epstein AN. Increased feeding in response to decreased glucose utilization in the rat and monkey. Am J Physiol 1969;217(4):1083-7.
87. Jenkins DJ, Wolever TM, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981;34(3):362-6.
88. Anderson GH, Woodend D. Consumption of sugars and the regulation of short-term satiety and food intake. Am J Clin Nutr 2003;78(4):843S-9S.
89. Venn BJ, Green TJ. Glycemic index and glycemic load: measurement issues and their effect on diet-disease relationships. Eur J Clin Nutr 2007;61 Suppl 1:S122-31.
90. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. Pediatrics 1999;103(3):E26.
91. Gunnerud U, Holst JJ, Ostman E, Bjorck I. The glycemic, insulinemic and plasma amino acid responses to equi-carbohydrate milk meals, a pilot- study of bovine and human milk. Nutr J 2012;11:83..
92. Ostman EM, Liljeberg Elmstahl HG, Bjorck IM. Inconsistency between glycemic and insulinemic responses to regular and fermented milk products. Am J Clin Nutr 2001;74(1):96-100.
93. Blundell JE, Green S, Burley V. Carbohydrates and human appetite. Am J Clin Nutr 1994;59(3 Suppl):728S-34S.
94. Feinle C, O'Donovan D, Horowitz M. Carbohydrate and satiety. Nutr Rev 2002;60(6):155-69.
95. Woods SC. Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. Am J Physiol Gastrointest Liver Physiol 2004;286(1):G7-13.
96. Woods SC, D'Alessio DA. Central control of body weight and appetite. J Clin Endocrinol Metab 2008;93(11 Suppl 1):S37-50.
97. Moran TH, Kinzig KP. Gastrointestinal satiety signals II. Cholecystokinin. Am J Physiol Gastrointest Liver Physiol 2004;286(2):G183-8.
98. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. Annu Rev Psychol 2000;51:255-77.
99. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50(8):1714-9.
100. Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988;81(2):442-8.
101. Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord 2001;25 Suppl 5:S63-7.
102. Richard D, Huang Q, Timofeeva E. The corticotropin-releasing hormone system in the regulation of energy balance in obesity. Int J Obes Relat Metab Disord 2000;24 Suppl 2:S36-9.
103. Broberger C, De Lecea L, Sutcliffe JG, Hokfelt T. Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the

rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. J Comp Neurol 1998;402(4):460-74.
104. Paz-Filho G, Wong ML, Licinio J. Ten years of leptin replacement therapy. Obes Rev 2011;12(5):e315-23.
105. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 1999;282(16):1568-75.
106. Lillioja S, Mott DM, Spraul M, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. New Engl J Med 1993;329(27):1988-92.
107. Ye J, McGuinness OP. Inflammation during obesity is not all bad: evidence from animal and human studies. Am J Physiol Endocrinol Metab 2013;304(5):E466-77.
108. Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 2008;57(5):1340-8.
109. Jakubowicz D, Froy O. Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes. J Nutr Biochem 2013;24(1):1-5.
110. Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. J Am Coll Nutr 2010;29(4):357-64.
111. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. Science 1984;224(4656):1392-8.
112. Muro Urista C, Alvarez Fernandez R, Riera Rodriguez F, Arana Cuenca A, Tellez Jurado A. Review: Production and functionality of active peptides from milk. Food Sci Technol 2011;17(4):293-317.
113. Pieniazek D, Grabarek Z, Rakowska M. Quantitative determination of the content of available methionine and cysteine in food proteins. Nutr Metab (Lond) 1975;18(1):16-22.
114. Zemel MB, Miller SL. Dietary Calcium and Dairy Modulation of Adiposity and Obesity Risk. Nutr Rev 2004;62(4):125-31.
115. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci USA 1997;94(26):14930-5.
116. Abou-Samra R, Keersmaekers L, Brienza D, Mukherjee R, Mace K. Effect of different protein sources on satiation and short-term satiety when consumed as a starter. J Nutr 2011;10:139.
117. Ma J, Stevens JE, Cukier K, et al. Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. Diabetes Care 2009;32(9):1600-2.
118. Bowen J, Noakes M, Clifton PM. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. J Clin Endocrin Metab 2006;91(8):2913-9.

119. Bowen J, Noakes M, Clifton PM. Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. Int J Obes (Lond) 2007;31(11):1696-703.
120. Stancliffe RA, Thorpe T, Zemel MB. Dairy attentuates oxidative and inflammatory stress in metabolic syndrome. Am J Clin Nutr 2011;94(2):422-30.
121. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr 2008;138(6):1047-52.
122. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D3 regulation of adipokine expression. Obesity 2007;15(2):340-8.
123. Legrand D. Lactoferrin, a key molecule in immune and inflammatory processes. Biochem Cell Biol 2012;90(3):252-68.
124. Dietitians of C, Canadian Paediatric S, College of Family Physicians of C, Community Health Nurses Association of C. The use of growth charts for assessing and monitoring growth in Canadian infants and children. Can J Diet Pract Res 2004;65(1):22-32.
125. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 2002;109(1):45-60.
126. Van Strien T, Frijters JE, Bergers G, Defares PB. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. Int J Eat Disord 2006;5(2):295-315.
127. van Strien T, Oosterveld P. The children's DEBQ for assessment of restrained, emotional, and external eating in 7- to 12-year-old children. Int J Eat Disord 2008;41(1):72-81.
128. Bellissimo N, Pencharz PB, Thomas SG, Anderson GH. Effect of television viewing at mealtime on food intake after a glucose preload in boys. Pediatr Res 2007;61(6):745-9.
129. Mollard RC, Wong CL, Luhovyy BL, Anderson GH. First and second meal effects of pulses on blood glucose, appetite, and food intake at a later meal. Appl Physiol Nutr Metab 2011;36(5):634-42.
130. Stubbs RJ, Hughes DA, Johnstone AM, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br J Nutr 2000;84(4):405-15.
131. Tamam S, Bellissimo N, Patel BP, Thomas SG, Anderson GH. Overweight and obese boys reduce food intake in response to a glucose drink but fail to increase intake in response to exercise of short duration. Appl Physiol Nutr Metab 2012;37(3):520-9.
132. Patel BP, Bellissimo N, Thomas SG, Hamilton JK, Anderson GH. Television viewing at mealtime reduces caloric compensation in peripubertal, but not postpubertal, girls. Pediatr Res 2011;70(5):513-7.
133. Bellissimo N, Thomas SG, Pencharz PB, Goode RC, Anderson GH. Reproducibility of short-term food intake and subjective appetite scores after a glucose preload, ventilation threshold, and body composition in boys. Appl Physiol Nutr Metab 2008;33(2):326-37.

134. Bellissimo N, Thomas SG, Goode RC, Anderson GH. Effect of short-duration physical activity and ventilation threshold on subjective appetite and short-term energy intake in boys. Appetite 2007;49(3):644-51.
135. Bozinovski NC, Bellissimo N, Thomas SG, Pencharz PB, Goode RC, Anderson GH. The effect of duration of exercise at the ventilation threshold on subjective appetite and short-term food intake in 9 to 14 year old boys and girls. Int J Behav Nutr Phys Act 2009;6:66.
136. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24(1):38-48.
137. Stratton RJ, Stubbs RJ, Hughes D, King N, Blundell JE, Elia M. Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding ad libitum. Eur J Clin Nutr 1998;52(10):737-41.
138. Raben A, Tagliabue A, Astrup A. The reproducibility of subjective appetite scores. Br J Nutr 1995;73(4):517-30.
139. Chaput JP, Gilbert JA, Gregersen NT, Pedersen SD, Sjodin AM. Comparison of 150-mm versus 100-mm visual analogue scales in free living adult subjects. Appetite 2010;54(3):583-6.
140. Polonsky K, Frank B, Pugh W, et al. The limitations to and valid use of C-peptide as a marker of the secretion of insulin. Diabetes 1986;35(4):379-86.
141. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 1996;50(3):253-64.
142. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41(3):368-77.
143. Medina-Bravo P, Meza-Santibanez R, Rosas-Fernandez P, et al. Decrease in serum adiponectin levels associated with visceral fat accumulation independent of pubertal stage in children and adolescents. Arch Med Res 2011;42(2):115-21.
144. Liljeberg Elmstahl H, Bjorck I. Milk as a supplement to mixed meals may elevate postprandial insulinaemia. Eur J Clin Nutr 2001;55(11):994-9.
145. Ruprichová L, Dračková M, Borkovcová I, Vorlová L. Determination of proteins in yoghurt. J Microbiol Biotechnol Food Sci 2012;1.
146. Gunnerud UJ, Ostman EM, Bjorck IM. Effects of whey proteins on glycaemia and insulinaemia to an oral glucose load in healthy adults; a dose-response study. Eur J Clin Nutr 2013;67(7):749-53.
147. Westphal S, Kastner S, Taneva E, Leodolter A, Dierkes J, Luley C. Postprandial lipid and carbohydrate responses after the ingestion of a casein-enriched mixed meal. Am J Clin Nutr 2004;80(2):284-90.
148. Nilsson M, Stenberg M, Frid AH, Holst JJ, Bjorck IM. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. Am J Clin Nutr 2004;80(5):1246-53.
149. Lan-Pidhainy X, Wolever TM. The hypoglycemic effect of fat and protein is not attenuated by insulin resistance. Am J Clin Nutr 2010;91(1):98-105.

150. Gunnarsson PT, Winzell MS, Deacon CF, et al. Glucose-induced incretin hormone release and inactivation are differently modulated by oral fat and protein in mice. Endocrinology 2006;147(7):3173-80.
151. Pende M, Kozma SC, Jaquet M, et al. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. Nature 2000;408(6815):994-7.
152. Arnberg K, Molgaard C, Michaelsen KF, Jensen SM, Trolle E, Larnkjaer A. Skim milk, whey, and casein increase body weight and whey and casein increase the plasma C-peptide concentration in overweight adolescents. J Nutr 2012;142(12):2083-90.
153. Eaton RP, Allen RC, Schade DS. Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. J Clin Endocrin Metab 1983;56(6):1294-300.
154. Ahren B, Thomaseth K, Pacini G. Reduced insulin clearance contributes to the increased insulin levels after administration of glucagon-like peptide 1 in mice. Diabetologia 2005;48(10):2140-6.
155. Rudovich NN, Rochlitz HJ, Pfeiffer AF. Reduced hepatic insulin extraction in response to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. Diabetes 2004;53(9):2359-65.
156. Meier JJ, Holst JJ, Schmidt WE, Nauck MA. Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. Am J Physiol Endocrin Metab 2007;293(3):E849-56.
157. El Khoury D, Brown P, Smith G, et al. Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin. Clin Nutr 2013.
158. Panahi S, Luhovyy BL, Liu TT, et al. Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults. Appetite 2013;60(1):154-61.
159. El Khoury D, Brown P, Smith G, et al. Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin. Clin Nutr 2014;33(1):29-38.
160. Ortinau LC, Culp JM, Hoertel HA, Douglas SM, Leidy HJ. The effects of increased dietary protein yogurt snack in the afternoon on appetite control and eating initiation in healthy women. Nutr J 2013;12(1):71.
161. Air EL, Benoit SC, Blake Smith KA, Clegg DJ, Woods SC. Acute third ventricular administration of insulin decreases food intake in two paradigms. Pharmacol Biochem Behav 2002;72(1-2):423-9.
162. Anderson GH, Saravis S, Schacher R, Zlotkin S, Leiter LA. Aspartame: effect on lunch-time food intake, appetite and hedonic response in children. Appetite 1989;13(2):93-103.
163. Braet C, Van Strien T. Assessment of emotional, externally induced and restrained eating behaviour in nine to twelve-year-old obese and non-obese children. Behav Res Ther 1997;35(9):863-73.

Appendices

Appendix 1 – Sample Size Calculation

Blood insulin levels of 11 normal weight 9-14 year old children were used to calculate this sample size as we found that using the same treatments we were able to detect a 45% increase in blood insulin levels over 120 min.

The equation used to calculate sample size when testing for the mean of a normal distribution (two-sided alternative), for within subject designs, is:

$n = [(z1\text{-}\alpha/2 + z1\text{-}\beta) \cdot \sigma/\Delta]2$

$\alpha = 0.05$, probability of Type 1 error

$\beta = 0.20$, probability of type II error

$Z\ 0.975 = 1.96$

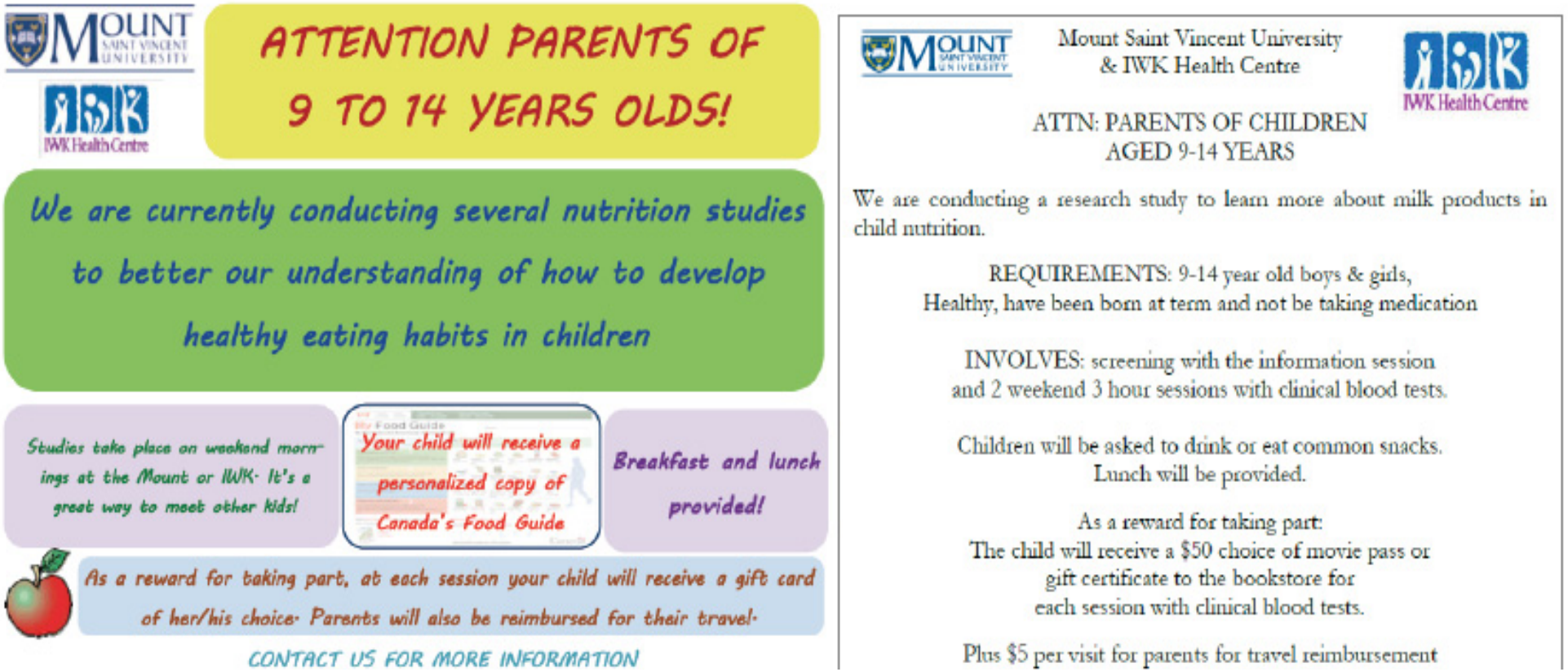
$Z\ 0.90 = 1.28$

$\sigma = 1.49$ μIU/mL

$\Delta = 5.71$ μIU/mL

$n = 7$

Appendix 2 – Recruitment Flyer



Appendix 3 – Telephone Screening Questionnaire

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 4 – Parental Consent Form

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

Study Information Sheet and Parent's Authorization Form

Investigators:

Name
Position
Department
Institution
Phone Number
Email

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 over weight. 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. 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 an investigator.

- 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: an investigator. You can also contact our Study Coordinator 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: _____

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

Appendix 5 - Child Assent Form

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:

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

_____
_____
_____

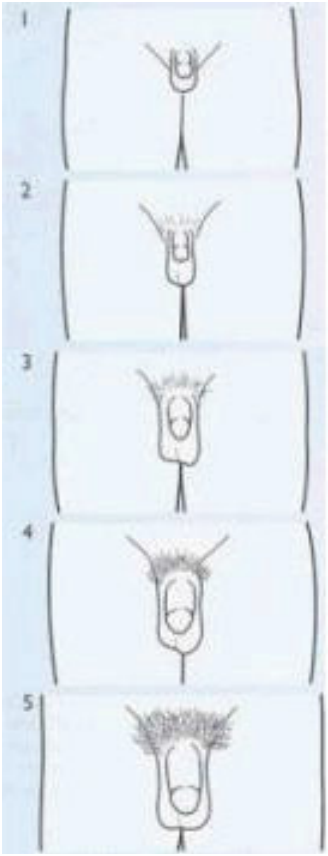
List each activity that you checked above in the "Activity" box below, check the months you did each activity and then estimate the amount of time spent in each activity.

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

Appendix 7 – Tanner Staging Self Administered 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

Appendix 8 – Self Administered 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 9 – 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

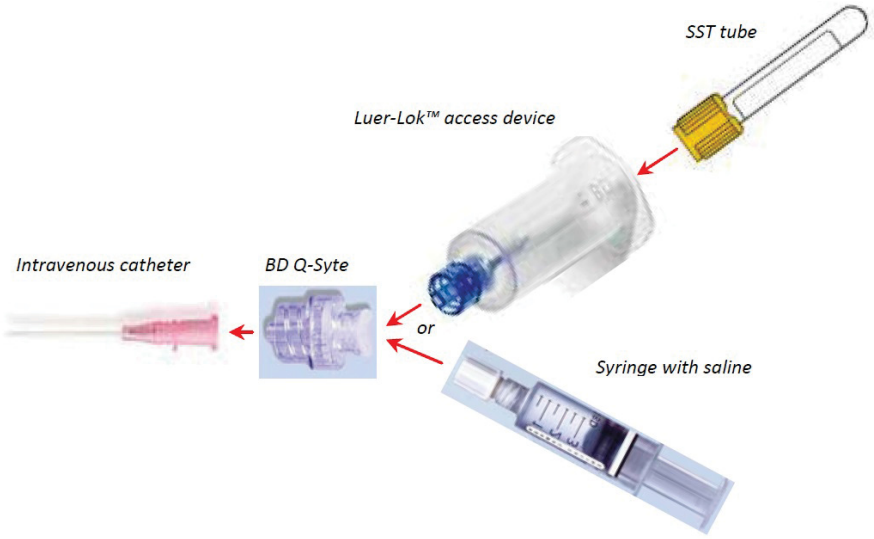
Appendix 10 - Nutrient Analysis for Dietary Treatments

Dietary Treatments for the study: Energy and Macronutrient Content:

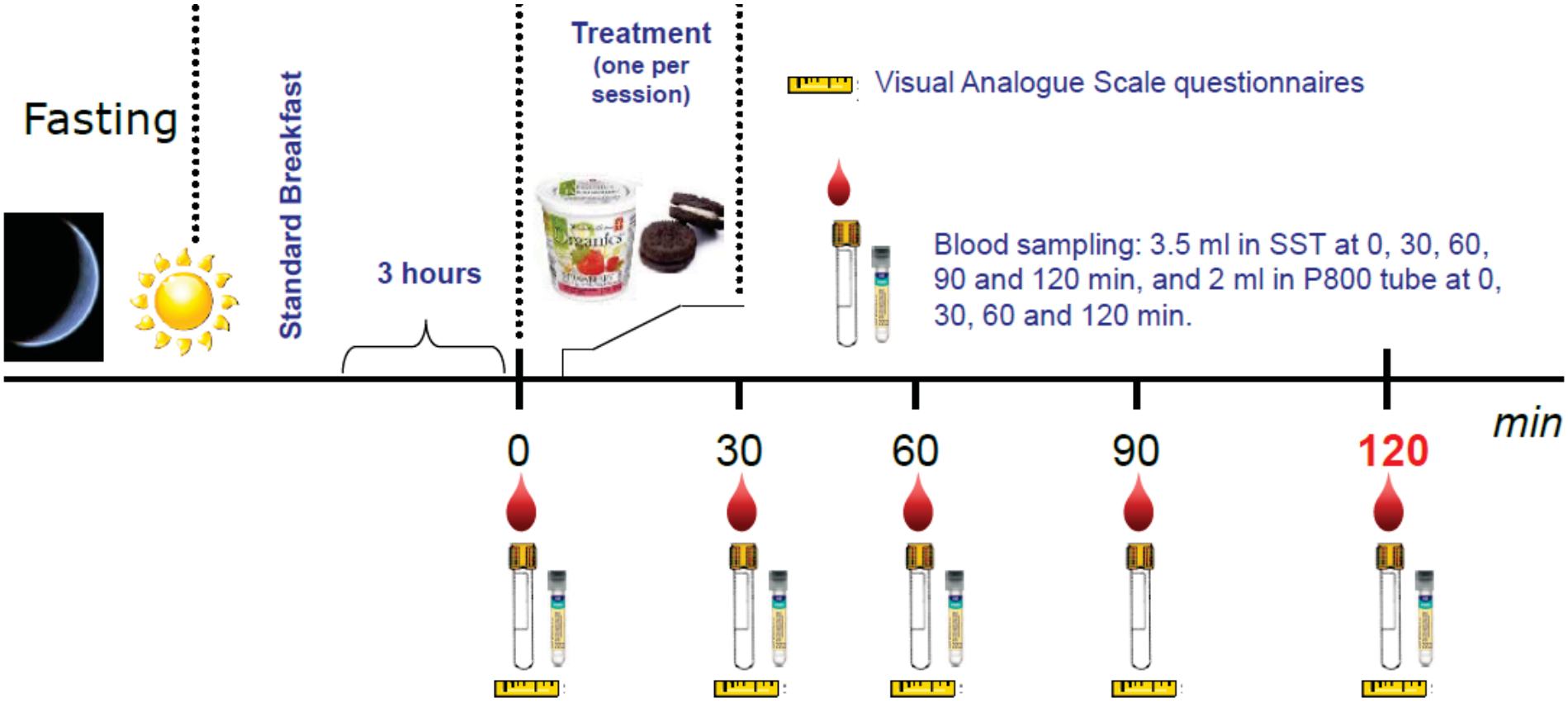
Energy and Macronutrients content per one treatment	*Yogurt (per 199 g)*	*Cookies (per 37.5 g)*
Energy (kcal)	*170.5*	*175.0*
Fat (total) (g)	*0*	*7.5*
Protein (g)	*17*	*1.3*
Total Carbohydrate (g)	*26.1*	*26.3*
Sugars (g)	*23.9*	*15.0*
Fibre (g)	*1.1*	*1.3*
Sodium (mg)	*62.5*	*212.5*
*Available carbohydrates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

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

Appendix 11 - Blood Collection System



Appendix 12 – Test Day Protocol



Appendix 13 – Sample Storage Protocol

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 – Example of Completed Visual Analogue Scale

Time = 120

Visual Analogue Scale
Motivation to Eat

DATE: Jan 1/13

NAME: AB-01

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.

25. How strong is your desire to eat?

Very WEAK — 17 mm — Very STRONG

26. How hungry do you feel?

NOT Hungry at all — 20 mm — As hungry as I have ever felt

27. How full do you feel?

NOT Full at all — 82 mm — VERY Full

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

NOTHING at all — 17 mm — A LARGE amount

Appendix 15 – Average Appetite Visual Analogue Scale

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 16 – Subjective Pleasantness and Sweetness Visual Analogue Scale

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 17 – Physical Comfort Visual Analogue Scale

Visual Analogue Scale
Physical Comfort

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

These questions relate to your "physical comfort" 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 well do you feel?

NOT well at all _____ VERY Well

Appendix 18 – 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]

166 Bedford Hwy Halifax Nova Scotia B3M 2J6 Canada
Tel 902 457 6350 • Fax 902 457 2174
www.msvu.ca

University Research Ethics Board

Certificate of Research Ethics Clearance

Effective Date	March 21, 2014	Expiry Date	March 20, 2015

File #:	2013-112
Title of project:	*The Effect of Dairy and Non-dairy Snack Products on Glycemic Regulation in Over Weight Children*
Researcher(s):	Brandon Gheller
Supervisor (if applicable):	Bohdan Luhovyy
Co-Investigators:	n/an/a
Version :	1

The University Research Ethics Board (UREB) has reviewed the above named research proposal and confirms that it respects the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* and Mount Saint Vincent University's policies, procedures and guidelines regarding the ethics of research involving human participants. This certificate of research ethics clearance is valid for a period of **one year** from the date of issue.

Researchers are reminded of the following requirements:	
Changes to Protocol	Any changes to approved protocol must be reviewed and approved by the UREB **prior** to their implementation. **Form: REB.FORM.002** **Info: REB.SOP.113** **Policy: REB.POL.003**
Changes to Research Personnel	Any changes to approved persons with access to research data must be reported to the UREB immediately. **Form: REB.FORM.002** **Info: REB.SOP.113** **Policy: REB.POL.003**
Annual Renewal	Annual renewals are contingent upon an annual report submitted to the UREB **prior** to the expiry date as listed above. You may renew up to four times, at which point the file must be closed and a new application submitted for review. **Form: REB.FORM.003** **Info: REB.SOP.116** **Policy: REB.POL.003**
Final Report	A final report is due on or before the expiry date. **Form: REB.FORM.004** **Info: REB.SOP.116** **Policy: REB.POL.003**
Unanticipated Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within seven (7) working days of the event. **Form: REB.FORM.008** **Info: REB.SOP.115** **Policy: REB.POL.003**
Adverse Research Event	Researchers must inform the UREB immediately and submit a report to the UREB within two (2) working days of the event. **Form: REB.FORM.007** **Info: REB.SOP.114** **Policy: REB.POL.003**

*For more information: http://www.msvu.ca/en/home/research/researchethics/policies/default.aspx

[redacted]

Dr. Daniel Séguin, Chair
University Research Ethics Board

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Approval - Full Board Review
March 05, 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
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