

Mount Saint Vincent University
Department of Applied Human Nutrition

The Effect of Dairy and Non-Dairy Products on Satiety and Food Intake in Children

Athena Chi-Yan Li

A Thesis Submitted in partial
fulfillment of the requirements for the
degree of Master of Science in Applied
Human Nutrition

December 2013
Halifax, Nova Scotia

© Athena Chi-Yan Li 2013

The Effect of Solid, Semi-Solid and Fluid Snacks on Food Intake and Satiety in Children

Athena C. Li

Master of Science in Applied Human Nutrition
Department of Applied Human Nutrition
Mount Saint Vincent University

The childhood obesity epidemic has become a growing health concern and one of the potential causes of this is the increase in the frequency of snacking among children. The objective of this study was to explore the short-term effects of dairy and non-dairy snacks on subjective satiety and energy intake in children. Methods: In a repeated-measures design, twenty-three normal weight (5th-85th BMI percentile) children (n=16 girls and 7 boys; aged 9-14 years old) were randomly assigned to one of five isocaloric (180kcal) treatments: deep fried chips (32g), strawberry Greek yogurt (200g), mini sandwich type cookies (39g), mozzarella cheese (63g) and 2% M.F. milk (346g). Following a 12hr fast, participants consumed a standardized breakfast two hours prior to each study session. Visual Analogue Scale questionnaires rating motivation to eat and flavour and texture preferences of the treatments were completed at: 0min (baseline), 15, 30, 45, 90 and 120 min. *Ad libitum* food intake was measured at a pizza lunch provided at 120min. Results: Food intake was 82kcal less following the mozzarella cheese treatment compared to 2% M.F. milk. ($P<0.05$) There was an effect of time ($P<0.0001$), but no treatment or time-by-treatment effect on subjective average appetite. Conclusion: A solid dairy snack (Mozzarella cheese) is effective in reducing food intake in children. Yogurt and milk suppresses food intake similarly to other popular non-dairy snacks, however, they provide better nutrient profiles. Funding source: Dairy Farmers of Canada.

Acknowledgements

“I can do all this through him who gives me strength”
Philippians 4:13 NIV

I would like to thank my thesis supervisor, Dr Luhovyy for supporting me, especially while I was completing my internships out of province. Your knowledge, patience, generosity and understanding have added immensely to my graduate experience and I could not have done this without your guidance. Dr Anini and Dr Nunes, thank you for taking the time and effort to be a part of my thesis committee. You have both reminded me about the importance of looking at a subject area from all possible aspects. Thank you Dr Anini for the lab space you generously provided for the analyses of the cortisol samples. I would also like to thank the Dairy Farmers of Canada for their financial contribution towards this study.

Thank you to the wonderful volunteers and research assistants in Dr Luhovyy’s Appetite Lab, especially Tove Armstrong. I could not have done this without your knowledge and guidance. Brandon Gheller, thank you for supporting me and for taking the time to teach me about SAS. You definitely made this process a lot less intimidating. A very special thank you goes out to Mary Gheller and Nadia Pabani for supporting me through this journey that we all started together. I am so grateful to have had the opportunity to share this experience with you both. Thank you to all my friends back home for your support and patience. The long distance telephone bills were worth every penny!

Last, but not least, thank you to my family for your love, encouragement and continued support. Thank you for never giving up on me and my dreams of becoming a dietitian and for teaching me to always work hard towards achieving my goals.

Table of Contents

Acknowledgements	2
List of Tables	6
List of Figures	7
List of Appendices	8
Abbreviations	9
Chapter 1 Introduction	10
Chapter 2 Literature Review	10
2.1 Obesity Prevalence and Trends in Canada.....	10
2.2 Food Intake Regulation.....	12
2.2.1 Central Food Intake Regulation	12
2.2.2 Peripheral Food Intake Regulation	13
2.3 Obesity Comorbidities	17
2.3.1 Cardiovascular Complications and the Metabolic Syndrome	17
2.3.2 Type 2 Diabetes Mellitus	18
2.3.3 Obesity into Adulthood.....	19
2.4 Possible Causes of the Obesity Epidemic	19
2.4.1 The Built Environment.....	20
2.4.2 Changes in Food Consumption Patterns	21
2.4.3 Soft Drink Consumption	23
2.4.4 Consumption of Snacks and Fast Food.....	23
2.4.5 Canadian Food Consumption Patterns	24
2.5 Possible Role of Dairy in the Prevention of Obesity	26
2.5.1 Milk Protein and Satiety	27
2.5.2 Casein vs. Whey.....	28
2.5.3 Dairy Products, Food Intake Regulation and Nutritional Implications.....	28
2.6 Cortisol's Role in Stress and Appetite	30
2.6.1 Special Considerations when Measuring Salivary Cortisol Levels	31
2.7 Tanner Staging	32
Chapter 3 Research Question and Hypothesis	33
3.1 Methods of Data Collection	33
3.1.1 Study Design.....	33
3.1.2 Treatments.....	34

3.1.3 Participants.....	34
3.1.4 Protocol.....	36
3.1.5 Ethical Considerations and Safeguards.....	39
3.2 Data Analysis.....	40
Chapter 4 Results.....	41
4.1 Subject Characteristics.....	41
4.2 Sensory Characteristics of the Treatments.....	43
4.3 Food Intake.....	45
4.4 Water Intake.....	45
4.5 Sodium Intake.....	46
4.6 Subjective Appetite Scores.....	48
4.7 Cortisol.....	49
4.8 Correlations.....	52
4.8.1 Subject Characteristics.....	52
4.8.2 Food Intake.....	52
4.8.3 Cortisol.....	53
Chapter 5 Discussion.....	54
Chapter 6 Limitations.....	63
Chapter 7 Conclusion.....	65
Chapter 8 Implications of the Study and Future Directions.....	65
References.....	66
Appendices.....	73
Appendix 1: Nutrition Information for Dietary Treatments.....	73
Appendix 2: Ethics Clearance from the University Research Ethics Board at MSVU.....	74
Appendix 3a Newspaper Advertisements.....	75
Appendix 3b Online Advertisements.....	76
Appendix 3c Posted Advertisements.....	77
Appendix 4a Telephone Screening Form.....	78
Appendix 4b Participant Exclusion List.....	79
Appendix 5a Recruitment Letter.....	80
Appendix 5b Parental Consent Form.....	81
Appendix 5c Participant Consent Form.....	86
Appendix 6a Puberty Questionnaire.....	87

Appendix 6b Tanner Staging Questionnaires	88
Appendix 6c Menstrual Cycle Questionnaire	90
Appendix 6d Physical Activity Questionnaire	92
Appendix 6e Dutch Eating Behaviour Questionnaire	93
Appendix 7a VAS Cover Sheet	95
Appendix 7b VAS Motivation to Eat.....	97
Appendix 7c VAS Physical Comfort	98
Appendix 7d VAS Sweetness	99
Appendix 7e VAS Pleasantness.....	100
Appendix 8 Detailed description of the method of saliva collection for salivary cortisol assay	101
Appendix 9 Nutrition Information for Pizza Meal.....	103
Appendix 10 Salivary Cortisol ELISA Procedure	104

List of Tables

Table 1: Subject Characteristics	42
Table 2: Effect of Treatments on Food Intake, Water Intake, Sodium Intake, Treatment Pleasantness and Sweetness, and Physical Comfort	78
Table 3: FI Correlations	53
Table 4: Cortisol Concentration Correlations	54

List of Figures

Figure 1: Treatment Pleasantness	44
Figure 2: Treatment Sweetness	44
Figure 3: <i>Ad libitum</i> Food intake	45
Figure 4: Water intake	46
Figure 5: Sodium intake	47
Figure 6: Subjective Appetite	48-49
Figure 7: Cortisol	50-52

List of Appendices

Appendix 1: Nutrition Information for Dietary Treatments	73
Appendix 2: Ethics Clearance from the University Research Ethics Board at MSVU	74
Appendix 3a Newspaper Advertisements	75
Appendix 3b Online Advertisements	76
Appendix 3c Posted Advertisements	77
Appendix 4a Telephone Screening Form	78
Appendix 4b Participant Exclusion List	79
Appendix 5a Recruitment Letter	80
Appendix 5b Parental Consent Form	81
Appendix 5c Participant Consent Form	86
Appendix 6a Puberty Questionnaire	87
Appendix 6b Tanner Staging Questionnaires	88
Appendix 6c Menstrual Cycle Questionnaire	90
Appendix 6d Physical Activity Questionnaire	82
Appendix 6e Dutch Eating Behaviour Questionnaire	93
Appendix 7a VAS Cover Sheet	95
Appendix 7b VAS Motivation to Eat	97
Appendix 7c VAS Physical Comfort	98
Appendix 7d VAS Sweetness	99
Appendix 7e VAS Pleasantness	100
Appendix 8 Detailed description of the method of saliva collection for salivary cortisol assay	101
Appendix 9 Nutrition Information for Pizza Meal	103
Appendix 10 Salivary Cortisol ELISA Procedure	104

Abbreviations

Δ - Change from baseline
AgRP - agouti-gene-related protein
ARC - Arcuate Nucleus
BMI - Body Mass Index
CART - cocaine-and amphetamine-regulated transcript
CCHS - Canadian Community Health Survey
CDC - The Centre for Disease Control and Prevention
CHS - Canadian Health Survey
CKK - Cholecystokinin
CNS - Central Nervous System
CVD - Cardiovascular Disease
DMH - Dorsomedial Nucleus
GI - Glycemic Index
GIT - Gastrointestinal Tract
GLP-1 - glucagon-like peptide 1
HPA - Hypothalamic-Pituitary-Adrenal Axis
HTN - Hypertension
LHA - Lateral Hypothalamic Area
NLSCY - National Longitudinal Survey of Children and Youth
NPHS - National Population Health Survey
NPY - Neuropeptide Y
OXM - Oxyntomodulin
PNS - Peripheral Nervous System
POMC - Pro-opiomelanocortin
PP - Pancreatic polypeptides
PVN - Paraventricular Nucleus
PYY -Peptide YY
SNS - Sympathetic Nervous System
T2DM - Type 2 Diabetes
TRH - Thyrotropin-releasing hormone
TSH - Thyroid-stimulating hormone
VAS - Visual Analogue Scales
VMH - Ventromedial Nucleus

Chapter 1 Introduction

The obesity epidemic is not only affecting the children of North America, but it has become a growing health concern for children worldwide, (1) especially for children in developed countries. (1, 2) The 2007 guidelines developed by The Centre for Disease Control and Prevention (CDC) are most often used to define the weight status of children. (3) Their guidelines are both age- and sex-specific and categorize those within the 85th to 94.9th percentile and those with a greater than 95th percentile of Body Mass Index (BMI) as overweight and obese, respectively. (3-5) BMI is defined as weight (in kg) divided by height (in m) squared and provides an accurate prediction of body fatness that is used to determine risks of comorbidities. (5) The cause and prevalence of obesity, the consequences of obesity and the prevention and treatment of overweight and obesity in children and adolescents has become a worldwide public health issue that is currently the focus of several research studies. (1, 6, 7)

Chapter 2 Literature Review

2.1 Obesity Prevalence and Trends in Canada

There have been several studies that have tested the validity of BMI to predict body fatness and it was found that in general, BMI can be a good indicator of body fatness in children and adolescents, (8-10) and therefore, BMI is widely used in population-based studies. (3, 8, 9) However, it should be noted that other measures of body fat should be used in combination with BMI as there are many factors that affect weight in adolescence. (3, 8, 10) Another limitation in the Canadian studies was related to the data used to calculate BMI. Three major longitudinal studies used by researchers are the 1996 National Longitudinal Survey of Children and Youth (NLSCY) and the 1996 and the 1998 National Population Health Survey (NPHS). (8, 11, 12). The concern regarding these surveys is that the participants used either self-reported or parent-

reported weight or height values. However, the validity of self-reported height and weight values has been tested in several studies. (3, 8, 9) There was one study that used data from the 2004 Canadian Community Health Survey (CCHS) and compared the BMI values to those of the 1978/79 Canadian Health Survey (CHS). (13) Both the CCHS and the CHS took direct measurements of height and weight of a representative group of Canadians from all provinces and therefore the data from this comparative study will be used to describe the rising obesity epidemic in Canada. It should be noted that these surveys did not take into count the children in the three territories (Yukon, Northwest Territories and Nunavut). This is partially due the low population level and the fact that Nunavut did not exist as a territory in 1978/79. (13)

In 1978/79 12% of children (age 2-17) were overweight and 3% were obese. This increased to 18% and 8%, respectively, in 2004. (13) This means that from 1978/79 to 2004, the number of overweight and obese children aged 2-17 years old increased from 15% to 26%. In this study, there were interesting discoveries about age-specific increases in obesity among Canada's children. Shields (13) found that the prevalence of overweight and obesity in children aged 2-5 years old remained virtually unchanged. However, the prevalence of overweight and obesity in those who were 12-15 years old doubled, from 14% in 1978/1979 to 29% in 2004, with the rate of obesity alone in this age group tripling, from 3% 1978/1979 to 9% in 2004. (13)

Another study used the data from the 1996 NLSCY study to explore the provincial trends of overweight and obesity in Canadian children age 7-13 years old. (12) While in general, across all provinces, there was an increase in the prevalence overweight and obesity in children, particularity concerning was the increase in rate of overweight and obesity in the Atlantic Provinces. (12) In 1996, the children living in the Atlantic Provinces (New Brunswick, Nova Scotia, Prince Edward Island and Newfoundland) were 4.35 and 1.45 times more likely to be

overweight or obese than children in 1981 or when compared to the rest of Canada, respectively. (12) Another study looked at the prevalence of overweight and obesity among Grade 5 students in Nova Scotia in 2003 and found that 32.9% were overweight with 9.9% being obese (6). This is particularly concerning as there are many well-known adverse effects of obesity in childhood on health in adulthood, however, before delving into the adverse effects of obesity, an introduction into food intake regulation in the body should be reviewed.

2.2 Food Intake Regulation

Food regulation is both a fascinating and complex process that involves an intricate network of hormones that influence several organ systems within the human body. The simplest way of looking at the regulation of food intake is by separating the actions of hormones that are centrally and peripherally regulated. (14)

2.2.1 Central Food Intake Regulation

Several parts of the brain are involved in the regulation of appetite and food intake. (15) The hypothalamus, located just above the base of the skull, right above the brain stem, plays a major role in the regulation of food intake and energy balance through its various control centres that are regulated by neuropeptides. (16) Neurotransmitters and hormones sent from various organ systems throughout the body are processed through the Arcuate Nucleus (ARC) of the hypothalamus which influences energy balance by processing information received about nutritional status. (15, 17) Within the ARC, there are two major populations of neurons responsible for relaying information that will inhibit or promote food intake. Neuropeptides that promote the intake of food are called orexigenic and include agouti-gene-related protein (AgRP) and neuropeptide Y (NPY). (17) Neuropeptides that suppress appetite are categorized as anorexic and include pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART). (17) These neuropeptides then send messages to the paraventricular nucleus

(PVN), the lateral hypothalamic area (LHA), the ventromedial nucleus (VMH) and the dorsomedial nucleus (DMH), which are all located within the hypothalamus. (17)

The PVN is responsible for relaying the information from the ARC to the endocrine system that in turn influences appetite and food intake. (15) The LHA is responsible for relaying information from the CNS to the rest of the body and when stimulated, will promote appetite. (15, 18) The VMH, best known for its role in balancing energy throughout the body, receives information from the ARC and transmits this information to the DMH and brain stem that, when stimulated, will decrease appetite. (15, 17, 18) The DMH proceeds to relay this information to the pituitary and thyroid glands. Thyrotropin-releasing hormone (TRH) is responsible for relaying the information from the hypothalamus to the pituitary and thyroid glands through its ability to stimulate the release of thyroid-stimulating hormone (TSH). DMH may also play a role in stabilizing the 24-hour rhythm of TSH levels. (15)

2.2.2 Peripheral Food Intake Regulation

Leptin, released into the circulation predominately by white adipose tissue (19), is best known for its role in appetite regulation. In obese leptin deficient mice and humans, leptin has been shown to promote weight loss, decrease body fat %, normalize serum glucose and insulin values, increase body temperature and decrease food intake. (20) When food is consumed, adipose tissues utilize glucose and lipids and release leptin which then binds onto receptors in the ARC suppressing appetite and reducing food intake. (21, 22) This is a slow, continuous process that is dependent on the body's energy stores. However, the serum level of leptin in overweight and obese individuals is actually elevated. Many studies have demonstrated that obese individuals are resistant to the actions of leptin. (15, 21)

Insulin, released by the pancreas, is the hormone responsible for maintaining blood glucose levels through its influence on the uptake of glucose in various muscle and organ cells throughout the body and is dependent on the sensitivity of the muscle or organ to the actions of insulin. (15, 21) Insulin works in a negative feedback loop in that elevated serum glucose levels above the normal results in the rapid release of insulin which will stop when serum glucose levels return to normal. (21) When compared to leptin, the action of insulin is rapid in that it is immediately secreted after the ingestion of food. (15) Insulin also plays a role in food intake by suppressing appetite hormones and neuropeptides, for example NPY, in the brain, specifically via the ARC, DMH and PVN. (15, 16) Serum levels of both insulin and leptin are directly proportional to the amount of adipose tissue stores in the body and both hormones work in the ARC to inhibit the orexigenic hormones NPY and AgRP. (23)

Pancreatic polypeptide (PP) is a hormone that is inversely related to fat stores throughout the body. (15) Pancreatic polypeptide show a specific circadian pattern with lower serum levels observed in the morning and highest levels observed in the evening, with spikes in serum concentrations following food intake that last approximately 6 hours. (15) PP has an anorexic influence on food intake by its influence on the brain, its role in regulating gastrointestinal tract (GIT) hormones and by delaying gastric emptying. (15, 24) The amount of PP that is released by the pancreas is related to the amount of calories consumed at a meal and is also elevated through the influence of several hormones within the GIT and by gastric distension. (15, 24)

Peptide YY (PYY), which is structurally similar to the PP hormone described above, is a hormone that is released by the distal GIT, which includes the ileum, colon and rectum. Unlike its relative PP hormone, PYY does not follow a circadian pattern, instead serum levels begin to rise 15 minute post-ingestion and reach a plateau 1-2 hours after food ingestion that remains

elevated for 6 hours. (15, 25) The amount of PYY released is directly correlated to the type of nutrients consumed with serum levels elevating the most after a high fat meal compared to a high protein or high carbohydrate meal. (26) Serum elevation is also related to the release of several other hormones within the GIT. (15, 27) Serum PYY levels are rapidly elevated after the ingestion of food which suggests that there is a neural pathway that causes the release of PYY prior to the ingestion of food as the rapid increase of PYY immediately after ingestion of food happens before the food enters the ileum. PYY influences food regulation by suppressing appetite through slowing gastric emptying and the suppression of the release of pancreatic and gastric juices and it also promotes fluid absorption in the small intestine. (15)

Ghrelin is a hormone that has received a lot of attention as it is the only gut hormone discovered to increase food intake. (27) It is released mainly in the stomach and the small intestine in response to fasting or caloric restriction. (15) The release of ghrelin also seems to be influenced by neural messages sent from the brain in anticipation of food as ghrelin increases appetite which increases food intake and therefore in return may cause an increase in body weight. (28) In rodents, ghrelin works to increase appetite by stimulating the release of AgRP and NPY, which are orexigenic hormones (29) and by increasing gastric emptying. (15, 27) Upon the consumption of a meal, serum ghrelin levels decrease, which therefore decreases appetite and slows gastric emptying, which is not seen in obese individuals and therefore may play a role in the cause of obesity.(28) Elevated ghrelin levels also seem to positively influence appetite scores in human studies. (15) For these reasons, an area of current research focuses on looking at reducing the bioavailability of ghrelin as a potential treatment for obesity. (15, 27)

Another hormone released by the stomach, pancreas and small intestine is glucagon-like peptide 1 (GLP-1), which is released after the ingestion of food. Serum GLP-1 levels are

inversely related to body mass and acts to inhibit food intake by slowing gastric emptying, increases insulin and suppresses the secretion of glucagon. (16, 17) The actions of GLP-1 to promote satiety and weight loss and to inhibit gastric distension are made possible by its influence on the brain stem. (15, 16, 30) GLP-1 shares receptors with Oxyntomodulin (OXM) which is released by the small intestine and is directly proportional to caloric intake and displays a daily pattern with peaks occurring in the evening and the lowest concentration displayed in the early morning. (15) OXM plays a role in the suppression of hunger and in turn, food intake, through its influence on the brain and its ability to decrease gastric motility and gastric secretions and it may also inhibit the release of ghrelin. (15, 24) The role of OXM requires further research into the specific mechanisms that occur in the body as a result of its release. (15)

Cholecystokinin (CKK) is a hormone released by the GIT, but is most often found in the proximal portion of the small intestine. CKK is rapidly released after the ingestion of food in response to fatty acids and protein and plays a role both peripherally and centrally. (15-17, 24) Its actions on the CNS include the regulation of rewards, behaviours, memory and satiety. (15, 17) Adding to the physiological actions of gastric distension that naturally occurs when food is ingested, CCK helps increase satiety by promoting the release of enzymes used to aid in digestion and by improving intestinal motility and reducing gastric emptying. This in turn will decrease both the size and duration of meals. (15, 17, 31) Due to its short half-life of 1-2 minutes, CKK is ineffective as an acute method of weight loss. However, since the actions of CKK are enhanced by leptin, it has potential to have a long-term effect on enhancing satiety. (15, 16)

2.3 Obesity Comorbidities

Studies looking at the adverse health effects of childhood overweight and obesity have paved the way to increased research into the prevention and treatment of obesity in childhood. This is in part, due to the recent rise in comorbidities of the Metabolic Syndrome seen in children that increases the chances of the development of cardiovascular disease (CVD), stroke and Type 2 diabetes mellitus (T2DM), which were historically seen only in adults. (6, 32, 33)

Comorbidities of the Metabolic Syndrome include abdominal obesity, high blood pressure, elevated fasting blood sugar levels, elevated triglycerides and low levels of HDL cholesterol. Someone with more than 3 of the above comorbidities is known to have the Metabolic Syndrome. (34)

2.3.1 Cardiovascular Complications and the Metabolic Syndrome

One classic study that explored the CVD risk factors in children and adolescents was The Bogalusa Heart Study. (35) While the data was based on a group of children age 5-17 years old who lived in Bogalusa, Louisiana from 1973-1994, it is a study that is often referenced because of the profound results in the effect of obesity in the development of CVD in children. It should be noted that this study was done in the late 90's, which was before the 2000 CDC update on their BMI guidelines for children. (5, 35) In this study, children with a BMI > 95th percentile were considered as overweight. (35) Today, with the updated CDC guidelines, these children are categorized as obese, (5) therefore for the purpose of this paper, obese and non-obese will be used to categorize the children in this study. The Bogalusa Heart Study found that obese children are at a higher risk of having risk factors of CVD compared to non-obese children. (35) More specifically, they found that obese children were 7.1 times more likely to have elevated triglyceride levels, 3.4 times more likely to have lowered HDL cholesterol levels and 12.6 times more likely to have elevated serum insulin levels. (35) Obese children are also more likely to

have high blood pressure compared to non-obese children. (35) Keep in mind that a child with three or more of the above mentioned comorbidities is at a greater risk of developing CVD, stroke and/or T2DM. Aside from the possible cardiovascular concerns that obesity places on children, obesity can also negatively affect the metabolic system.

2.3.2 Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is another rising concern in children today. (33, 36, 37) A study completed in 2010 looked at the incidences of newly diagnosed T2DM in Canadian children from 2006 to 2008 and found that the rate of T2DM is on the rise. (33) It was found that the general, the age of diagnosis of T2DM was 13.7 ± 2.5 years with 58% of newly diagnosed cases being female. (33) It was also found that of those 18 years old and younger, 8% of newly diagnosed T2DM was found in individuals who were less than 10 years old. (33) The authors also found that of the children newly diagnosed with T2DM, 37% presented with one of the 6 comorbidities including obesity, dyslipidemia, hypertension, non-alcoholic fatty liver disease, kidney disease and polycystic ovary syndrome and 13% presented with 3 or more of these comorbidities. (33) This is alarming as it shows not only the increasing incidences of the disease in the Canadian pediatric population; it also paints a picture of the changing face of T2DM in this population. It is well known that a major risk factor for T2DM is obesity, with an estimated 85% of newly diagnosed American children being classified as overweight or obese at the time of diagnosis. (37) Due to the advances in medicine, children today are at risk of developing cardiovascular and metabolic complications in early adulthood and therefore will be sick for a longer period of time throughout their lifetime. This leaves them more vulnerable to the possible adverse effects of CVD and metabolic disorders that develop at a younger age compared to those in earlier generations.(32)

2.3.3 Obesity into Adulthood

Overweight or obese children tend to become overweight or obese adults. (4, 10, 38)

There have been a few studies that have looked at the relationship between childhood and adulthood BMI and found that there was a correlation that differed depending on the age and sex of the child. In general, overweight or obese children under the age of 2 were not at risk of becoming an overweight or obese adult, (4) however, the chances of becoming an overweight or obese adult increased as the age and BMI of the child increased. (10, 38) Also, the relationship between childhood BMI and adulthood BMI was found to be stronger in males than in females. (10) It is a widely accepted notion that the prevention and treatment of overweight and obesity is more effective in children and adolescents, as they have had less time to develop unhealthy lifestyle choices compared to young adults and there are more intervention opportunities for children and adolescents compared to young adults. (39) Adolescence is also a time in life where behaviours, especially eating habits, are formed. (40, 41) It is known that adults, in general, tend to gain weight and rarely is this weight lost to return to a normal BMI range, therefore, prevention of weight loss is more effective than the treatment of overweight and obesity. (13) This justifies the measures for preventing and treating childhood overweight and obesity.

2.4 Possible Causes of the Obesity Epidemic

While it is often agreed that the general cause of obesity is related to the imbalance between energy intake and energy expenditure, this is only part of the picture regarding the causes of overweight and obesity. In general, obesity is the long term effect of energy intake exceeding energy expenditure; however this imbalance as the only cause of obesity would be too simplistic. (42) Instead, many other factors, such as the built environment, culture and, to a certain degree, genetics all play a role in overweight and obesity in children. (1, 7, 42) The increase in sedentary activity and the decrease in physical activity or manual labour have been at

the forefront of the cultural reasons for the rise in obesity, as are current eating habits and food trends. (2, 42)

2.4.1 The Built Environment

An obesogenic environment is often used to describe the environment of the North American culture as it often promotes sedentary behaviour and food choices of low nutritional quality. As mentioned earlier, the culture and environment of North America likely play a role in the recent rise in obesity. (1, 7, 40, 42) An obesogenic environment includes the physical and social environment that has been developed in North America that promotes weight gain. For example, the lack of park space in urbanized cities means that children and adolescents have less open space to play in. (7) Access to recreation centres is also included in the built environment as is public transportation. Most urbanized cities have developed public transportation systems that make for less opportunity for walking and cycling. (7) The increase in sedentary activity, such as video games, television, computers and the internet have played a major role in the decrease in physical activity seen especially in children and adolescents. (7) The change in the school system is also part of the obesogenic environment that is often blamed for the rise in obesity in North America. (7)

The lack of fresh fruits and vegetables available in public schools as well as the use of vending machines and their possible role in the change of nutrition environment in North America is currently a topic of interest. (43) In the last few years there have been numerous debates about the use of vending machines in public schools and how school boards can provide healthier options for children and adolescents while in school. (43) Recently, the Ontario government began regulating the food in school cafeterias; however there has been a lot of resistance from the students, so cafeterias are beginning to see a loss in sales as adolescents have

begun to find off-site lunch options. (44) Gerard Kennedy was the Minister of Education in 2004 when he wrote a report on the new healthy guidelines on the foods sold in school cafeterias where he cited a review by the Dietitians of Canada that looked at the foods and beverages sold in school vending machines. In Kennedy's report (45) it says that in schools where soft drinks and milk were both offered, milk consumption dropped by 30%. It was also found that children that consumed soft drinks also had, on average, one less serving of milk per day. (45) This leads to an important change in the food environment that may be contributing to the rise in obesity, which is the consumption of more fast-foods, convenience foods and snacks and the decrease in the consumption of whole foods.

2.4.2 Changes in Food Consumption Patterns

Recently, there has been greater focus placed on the food group consumption patterns of children and adolescents in North America as a way to discover if there is a connection between the changes in food group consumption patterns and obesity. It should be noted that while most of these studies are based on the general American population, most Canadian studies have shown that the consumption patterns between Americans and Canadians are very similar. (43) One major change that has occurred is the decrease in the consumption of whole foods, for example dairy, fruits and vegetables and whole grains, and the increase in the consumption of quick, convenient "mixed" foods that are energy dense but have a low nutritional value. Cavadini, *et al.* (40) found that from 1965 to 1996, American adolescents age 11-18 years old consumed less whole raw fruits, non-potato vegetables and dairy. This leads to a less than optimal intake of essential nutrients needed for the growth and development of children and adolescents, for example fibre and calcium. (40) Also, a decrease in energy from fat intake and an increase of energy from carbohydrates was also observed. (40, 46) The increase of carbohydrates came mainly from mixed dishes, for example, macaroni and cheese and pizza and

fried potatoes, which has seen a steady increase in consumption since 1965. (40, 46) Research suggests that the food group consumption pattern of North American children and adolescents have moved from a diet of predominately whole foods, to a diet high in carbohydrates and fat which are of lower nutritional quality. (40, 46) This major change in food group consumption patterns have occurred mainly due to cultural and environmental changes that have occurred within the last 40-50 years. (46, 47) Not only is the changes in food group consumption a concern in this population; there has also been a huge change in the types of foods that are consumed.

Today, in North America, the need for convenience food compensates for the lack of opportunities to prepare home cooked meals. A greater portion of families today have two working parents who may not have the time to cook a well-balanced meal and therefore more individuals and families are relying on fast food, restaurants and convenience foods to suite their busy, on-the-go lifestyle. (40, 47) Not only has there been changes in the type of food that is consumed, the portion size of many foods have also increased since the 1970's. (47) The reason for the concern with the changes in the food consumption patterns in North American children within the last 40-50 years is related to its correlation to the rise in obesity.

The rapid increase in the consumption of fast and convenient foods, which are usually less-nutrient dense foods and are on average higher in calories, sugars and salt, may play a huge role in the rise in obesity in children and adolescents. (47-49) A study that explored the factors that affect nutritional intake of adolescents in grades 7-12 found that adolescent males and females who consumed a meal at a fast food restaurant ≥ 3 times a week had a 40% and 37% increase in energy intake, respectively, compared to those who did not eat a meal at a fast food restaurant. (50) In the same study, it was also found that there was a significant relationship

between increased fast food consumption and decreased consumption of fruits and vegetables, grain products and dairy products. (50) The consumption of soft drinks and obesity is also another relationship that has received much attention.

2.4.3 Soft Drink Consumption

One study looked at the changes in the consumption of soft drinks and found that it may be correlated to obesity. (48) In the last 40-50 years there has been an increase in both soft drink consumption, and obesity. (48, 49) There also tends to be a positive relationship between the amount of soft drinks consumed and age, therefore, soft drink consumption may be inversely related to the quality of the diet. It was shown that the increase in soft drink consumption observed in children and adolescents, was accompanied by the decrease in milk consumption. (46) For example, in adolescent females between the late 1970's to 1994, the consumption of soft drinks increased 65% in adolescent females, while within the same time period the consumption of milk decreased from 72% to 57% in adolescent females. (46) Similar findings in both males and females have been seen in several other studies. (48, 49)

2.4.4 Consumption of Snacks and Fast Food

Several studies have shown that the increased consumption of snack foods and fast foods in children and adolescents have contributed to the decrease in the nutritional quality of foods consumed by children and adolescents, which may be contributing to the rise in obesity. (46, 49, 50) It is presumed that the energy consumed from snack foods and fast foods is replacing the energy that would have been consumed from fresh fruits and vegetables, whole grains and dairy products. This is concerning as it has been shown that children and adolescents do not compensate for their energy intake from snacks. (51) Instead, the calories from snacks are in addition to calories and macronutrients from meals. (47, 51) One thing that should be noted is that the calories from individual snacks have stayed relatively stable. (52) The change is in the

frequency of snack consumption in children and adolescents. Jahns, *et al.* found that there was an increase in the snacking frequency in children 2-18 years old from 77% to 91% from 1977-1996, but most of this increase was seen between in the latter years, 1989 to 1996. (52) Along with this general increase in the frequency of snack consumption, the total energy intake from snacks increased by 30% within the same time period. (52) By 1996, approximately 25% of the total energy intake in children and adolescents was from snacks alone, with adolescent males and females age 12-18 years old consuming the greatest amount of calories from snacks. There is a need to study more about food group intake and weight in children and adolescents as there is very little research available in this topic area. (48) This is especially true in Canadian children and adolescents.

2.4.5 Canadian Food Consumption Patterns

While most of the available data on the food patterns of Canadian children and adolescents is either from Ontario or collected specifically on the Aboriginal population, it should be noted that in general, the patterns of Canadian children and adolescents are similar to those of American children and adolescents. Gilbert *et al.* (51) found that the energy from after-school snacks alone provided approximately 13% of total energy intake in children age 2-18 years old, and this was only on the after-school snacks consumed Monday to Friday from 3pm to 6pm. It is actually estimated that the energy consumed from snacks in children is greater than the energy consumed at breakfast or lunch. (43) One can only imagine how much energy from snacks contributes to a child's total energy intake on an average day. It goes without saying that increased snack consumption increases total energy intake, however, it was interesting that the consumption of a high calorie afternoon snack also increased intake at dinner compared to those who did not consume an afternoon snack. (51)

With respect to the specific types of snacks consumed, it is interesting to see that there is a difference between American and Canadian snack choices. A market analysis by Agriculture and Agri-Food Canada (53) shows that chips and crisps are the most popular snacks in America, and this trend is expected to continue until at least 2015, however it should be noted that this analysis was completed on the general American population, not just children. In Canadian children and adolescents, the opposite snack preference is seen. The most popular after school snack among Canadian children age 2-18 years old are sweets, with cookies and granola bars providing the most energy out of all other after school snacks as well as being the most preferred after school snack. (51) It is interesting, however, that there seems to have been a revival in the intake of yogurt and cheese in the past few years. A report that looked at the Canadian food preferences in 2008 revealed that the interest in yogurt, and its use as a functional food, has helped the value of yogurt grow by 11% in 2008. (54) In the same report, the decrease in milk consumption was seen, but this decrease was accompanied by an increase in cheese and cream product consumption. (54) It should be noted that this market analysis refers to the general Canadian population, but this is promising as there is a clear connection between the availability of healthy foods at home and lowered incidences of obesity. (7, 43)

Canada's Food Guide to Healthy Eating suggests that children age 2-18 years old should consume 2-4 servings of dairy products per day, depending on age. (55) A study that looked at the food consumption patterns of children age 8-10 years old in Hamilton, Ontario found that 58.4% consumed less than the suggested 3-4 dairy food servings per day. (56) This decrease in dairy intake is similar to results found in several American-based studies. (57, 58) The possible relationship between dairy intake and body weight is also of interest. (59-61)

2.5 Possible Role of Dairy in the Prevention of Obesity

Recognizing the trend in decreased dairy intake in children, researchers have taken this opportunity to explore the possible connection between dairy and/or calcium intake and body weight. (58, 60, 61) Only a handful of research has been done in children and adolescents and that the studies that show a strong correlation between dairy/calcium intake and weight loss were mostly explored in adults. (57, 58, 61) In general, the relationship between dairy/calcium intake and body weight is inconclusive.

When Berkey *et al.* used data from the Growing Up Today Study to explore the relationship between the consumption of milk, calcium and dietary fats and the change in BMI over time in children age 9-14 years old, they found that those who consumed greater than 3 servings of milk per day had greater weight gain than those who consumed less than or equal to 0.5 servings of milk per day. (62) One interesting finding from this study was that there was no association between dairy fat and weight gain. (62) Using data from the Framingham Children's Study, Moore, *et al.* found that high dairy consumption (approximately 2.01 servings for girls and 2.84 servings for boys) at 3-6 years old was associated with lower BMI values and skin-fold measures 7 years later, after adjusting for energy and saturated fat. (63) Similar to the findings of Berkey, *et al.* Moore, *et al.* also found that those who consumed the greatest amount of dairy servings per day also had a higher intake of fat and calories. (63) However, compared to those who consumed the least amount of dairy products per day (approximately 1.09 servings for girls and 1.38 servings for boys), those who had consumed the most servings of dairy per day had lower BMI values and skin-fold measures 7 years later, after adjusting for energy and saturated fat. (63) It should be noted that the Framingham Children's Study was conducted from 1987 to 1999, (63) which is about the same time that the increase in the frequency of the consumption of

snacks really started to take hold in North American children. A clinical trial by Keishadi *et al.* (60) explored the relationship between dairy intake, obesity and signs and symptoms of the metabolic syndrome in obese Iranian children and found that a dairy-rich diet may help prevent symptoms of the metabolic syndrome in prepubescent, obese children. (60) However, it should be noted that this study only looked at Iranian children who consume a very different diet from that of a typical North American child. These studies stress the fact that the answer to the possible causes of obesity in children is not as simple as the consumption of a specific food or nutrient, for example, the relationship between dietary calcium and weight loss.

While the relationship between calcium and weight loss/maintenance has also been explored as a possible “cure” for obesity, (57) it should be noted that while calcium may play a role in weight loss/maintenance, it should not be seen as the magical answer to obesity. (57) If obesity was as easy as increasing intake of a single nutrient, there would be no need for the research in this topic area that is currently happening. Before delving into the satiating effects of dairy products, it should be noted that satiety refers to the feeling of fullness that prevents further food intake after the consumption of a meal, whereas satiation refers to the action of food intake to eventually reach satiety that occurs during a meal. (16, 64)

2.5.1 Milk Protein and Satiety

Among the macronutrients, the satiating effect of protein is greater than that provided by fats or carbohydrates, and is dependent on the type of protein. (65, 66) Therefore the focus of this proposal will turn to the properties of milk proteins that promote satiety. Cow’s milk is made up of two major classes of proteins, casein and whey, which make up approximately 80% and 20%, respectively, of the proteins found in milk. A study by Anderson *et al.* (67) compared the effect of whey, soy and egg protein on food intake at a meal 1 and 2 hours later and found that whey

protein, compared to soy and egg proteins, was the best at suppressing food intake at a meal 1 and 2 hours later. Another study looked specifically at the differences in whey and casein on satiety in 16 young adults and found that total food intake was less in the whey treatment when compared to the casein treatment. (65) In a classic study by Mellinkoff *et al.* (68) it was found that elevated serum levels of amino acids, the final product of protein digestion, suppressed appetite, therefore the kinetics of casein and whey protein digestion can be used to explain the differences in the satiating properties of milk proteins.

2.5.2 Casein vs. Whey

Gastric juices in the stomach coagulate with casein to form somewhat of a gel that is slowly emptied into the small intestines. (66) This, however, does not happen with whey proteins, and therefore casein protein is often categorized as a “slow” protein because it suppresses gastric emptying. (69) Whey on the other hand is often called a “fast” protein because it is digested in the small intestines faster than casein, and therefore elevates the serum levels of amino acids faster than casein would. (65, 69) Whey has the potential to raise serum amino acid levels as fast as 30-40 minutes after ingestion and these levels remain sustained for up to 2 hours post ingestion, (16) therefore whey proteins provide better short-term satiety than casein. However, since casein slows gastric emptying, it provides a sustained elevation of serum amino acids and therefore provides long-term satiety. (65)

2.5.3 Dairy Products, Food Intake Regulation and Nutritional Implications

With respect to the hormonal controls that dairy products have on satiety, there is evidence that dairy proteins may play a role in promoting satiety, and therefore decreasing appetite and food intake. (16) While only casein and whey proteins were reviewed above, it should be noted that both whey protein and casein are complex proteins composed of individual units, all of which produce a different effect on the hormones and neurological processes that

affect food intake regulation. (16) Milk proteins have the potential to synergistically stimulate the release of GLP-1 and decrease ghrelin in the blood, inducing satiety. With respect to whey and casein, whey proteins stimulate the release of CCK greater than casein proteins, which again, helps promote satiety. (16) Aside from the positive influences of dairy products on food intake regulation, they also provide the body with several essential nutrients that promote a healthy, nutritious diet. (40)

Dairy products contain several minerals, such as calcium, phosphorus, magnesium, zinc and potassium, which not only provide the body with antioxidants, these minerals are also essential for the growth, development and maintenance of healthy teeth and bones. (16, 49) While dietary fats are often blamed for being one of the reasons for the obesity epidemic, it should be noted that not all fats are detrimental to health. For example, the absorption of the fat-soluble vitamins A, D, E and K is enhanced by the consumption of fatty acids. (16) Aside from the nutritional aspects of fat consumption, fatty acids also play a role in food intake regulation through its ability to stimulate the ileal brake. The ileal brake is a negative feedback mechanism within the distal portion of the small intestine that aids in delaying gastric emptying and gastric motility, thus allowing for the slower digestion of food products and the suppression of appetite. (16) Several other fats found in dairy products have been shown to improve serum cholesterol levels and therefore dairy products may play a role in the protection against weight gain and CVD, however, more research in this topic area is required in this topic area to confirm these findings. (16) For these reasons, fat-free dairy products may not provide the same health properties as higher-fat dairy options.

Lactose, the intrinsic sugar found in dairy products, is a disaccharide comprised of glucose and galactose. Lactose has a lower glycemic index (GI) than sucrose which means that

lactose is digested and absorbed at a slower rate than sucrose, therefore inducing a lower insulin response compared to glucose and allowing for greater control in serum glucose levels. (16, 70)

While there are many epidemiological studies that have looked at the relationship between dairy intake and obesity and provided inconclusive results due to many confounding factors, (60, 61) there is a lack of acute short-term studies, especially with children, investigating the effect of dairy ingestion on food intake regulatory system and factors of metabolic health.

2.6 Cortisol's Role in Stress and Appetite

The hypothalamic-pituitary-adrenal (HPA) axis is a hormonally regulated system that responds to stress in the body. (71, 72) Stress response happens through the interactions of the Sympathetic Nervous System (SNS) and the HPA axis. (71) In acute stress, the SNS releases epinephrine and norepinephrine which create the “fight-or-flight” response, whereas the HPA axis is activated as a slower response to stress with the end result being the release of cortisol into the blood stream. (71, 73) In healthy males, the administration of glucocorticoids increases *ad libitum* food intake, thereby ensuring that the body has enough fuel in preparation of the stressor. (72, 74) Increased levels of cortisol in women have been shown to increase caloric intake after a stressor is applied. (75) One theory to explain this is related to the body's response to lowered secretion of insulin and elevated serum levels of glucose to be used as fuel (72). This can trick the brain into thinking that body cells are starved of glucose, therefore increasing appetite which may in turn cause overeating.

In obese individuals, it has been shown that the mechanism responsible for clearing cortisol is enhanced, which causes the HPA to overcompensate, thus ensuring that cortisol homeostasis is maintained. (76) It has also been shown that men with Type 2 Diabetes Mellitus

(T2DM) had elevated levels of cortisol secretion and decreased HPA axis actions which then lead to increased consumption of high fat, salt and sugar food items, compared to individuals without T2DM. (77) Therefore, another theory is that elevated cortisol levels, seen in those who are obese and in individuals with T2DM (76, 77), may actually play an indirect role in appetite. When cortisol is released into the bloodstream, it creates a cascade effect in that several other hormones that plays a direct role in appetite and energy intake, for example leptin, NPY and cytokines, are also released into the bloodstream. (75, 78) For this reason, not only can cortisol be used to help measure stress levels in children; it can also be used to help measure appetite.

2.6.1 Special Considerations when Measuring Salivary Cortisol Levels

Due to the difficulty in collecting serum cortisol samples from children and infants, there is less known about the circadian pattern of cortisol in this population. It should also be noted that the differences in salivary cortisol levels in men and women are well documented, but this pattern has yet to be established in children. (79-81) In general, cortisol exhibits a predictive 24-hour (circadian) rhythm and a diurnal variation in humans. (81) Cortisol concentration is usually at its highest 20-30 minutes after waking up and its lowest at midnight, with slight elevations at mid-day, which is likely in response to the consumption of lunch. (80, 81) It has also been shown that plasma and serum cortisol concentrations respond to macronutrient composition. (76, 82) In a study by Stimson, *et al.* it was found that plasma cortisol concentrations rose following a high carbohydrate (140.5g carbohydrate) meal, high protein (19.7g protein) meal and a high fat (59g fat) meal, compared to a placebo meal in normal weight men. (82) It was found that after the high protein and high fat meals, plasma cortisol concentrations peaked at 45 min following the meal, but plasma cortisol concentrations following the high carbohydrate meal peaked after 75 min. This study also showed that the mechanisms causing the elevation of plasma cortisol following the different macronutrient meals were different. (82)

In a study by Tornhage, (80) it was found that the median cortisol concentration in the morning (between 8-9am) was lower in children age 7-9 years old compared to 10-12 year old children. Tornhage,(80) also compared morning cortisol concentrations between children and adults and found that children age 7-15 years old had lower median cortisol concentrations than adults. A study by McCarthy, *et al.* established normative salivary cortisol values in children age 4-10 years old as well as found that a change in cortisol concentration occurred 20 minutes after a stressor, in this study the insertion of an IV needle, was applied. (81) A problem with collecting salivary cortisol in the laboratory setting is that the environment may serve as a form of stress that can elevate the cortisol levels.(71) Another consideration is the effect of pH on saliva that may affect the efficacy of the assay used to measure the amount of cortisol in a sample, for example, saliva with a lower pH level may result in a false high cortisol sample. (71)

Another consideration that is not well understood is the effect of sex hormones, released during puberty, on cortisol levels. (71) Netherton *et al.* (83) found that there was a 20-30% increase in morning cortisol concentrations in adolescent females aged 8-16 years old who were in the mid- to post-pubertal stages compared to males in the same stage of puberty. This difference was not seen in adolescents who were in the pre- to early-pubertal stages. (83) The rapid change in adolescents during puberty makes it hard to do research on hormones in this population and as a result, there is very little known about hormones in this population. (84)

2.7 Tanner Staging

Pubertal development might be a factor influencing the eating behaviour and food intake regulatory system. (85, 86) There are main 3 phases of puberty that starts with an accelerated growth spurt, a deceleration, and the last stage is the cessation of puberty. (87, 88) It is generally agreed that the stages of puberty do not correspond to a specific age, however, the timing of what

happens generally coincide with the stages of puberty. (88) For example, the general flow of puberty for girls starts with breast development and ends with menarche. (89) In general, girls tend to enter the acceleration phase of puberty at an earlier age, ranging from 9.7-13.3 years old, where boys enter the acceleration age on average at 11.7 -15.3 years old. Rapkin *et al.* (90) found that in general, Tanner Staging was highly correlated to corresponding hormone levels and were in agreement with physician ratings..

Chapter 3 Research Question and Hypothesis

The purpose of this study was to explore the short-term effects of dairy products (milk, yogurt and cheese) on subjective satiety and energy intake at a test meal 2 hours later, compared to popular snack foods (cookies and chips) in normal, overweight and obese children age 9-14 years old. It was hypothesized that the consumption of dairy products will provide greater feelings of subjective satiety and decreased energy intake at a test meal 2 hours later.

3.1 Methods of Data Collection

3.1.1 Study Design

This study followed a preload paradigm using a repeated-measures design where the study participants received all treatments in a randomized order in order to decrease the daily variations that exist within subjects. (64) Twenty three normal weight children aged 9-14.5 years old attended 5 morning sessions with one week apart between the sessions. A sample size of 17-26 participants is sufficient to detect a difference of 500kJ with a power of 0.8 and was similar to the sample sizes used in previous studies with children. (91-93) During these sessions the children randomly received one of the five treatments, therefore this study was a randomized open-label clinical trial.

3.1.2 Treatments

Since energy intake was the main study interest, all treatments in this study were standardized to 180 kcal. The treatments were: (a) 2% M.F. milk (Farmers Cooperative Dairy Ltd.), (b) Strawberry 0% M.F. Greek Yogurt (Liberté Inc), (c) Cheese (Cheestrings Marbelicious Mozzarella Cheese, Black Diamond, Parmalat Canada Inc), (d) Mini Oreo Mr. Christie's Cookies (Kraft Canada), (e) Potato chips (Lay's Classic, Frito Lay Canada). A table with the weight and macronutrient information of the treatments is provided in **Appendix 1**. All treatments were given in a randomized order as determined by the Balanced Block Randomization function using SAS (version 9.2, SAS Institute Inc. Cary, NC)

3.1.3 Participants

The study was conducted according to the Declaration of Helsinki guidelines (94) and all procedures involving human participants were approved by the University Research Ethics Board at MSVU. (**Appendix 2**) Written informed consent was obtained from parents and assent forms were filled out by the children at the screening session.

Children were recruited through advertisements in local newspapers, (**Appendix 3a**) (The Coast and Metro Halifax), online advertisements (**Appendix 3b**) through <http://hrmparent.ca/> and by word of mouth. Upon approval by facility managers, advertisements were also posted on community bulletins (**Appendix 3c**) in facilities frequented by families and young children, for example the YMCA, the Dalplex, the Dartmouth Sportsplex and the Halifax Forum, just to name a few.

Prior to acceptance into the study, the child and/or their parent(s) were screened via telephone and email using the Telephone Screening Questionnaire (**Appendix 4a**) to determine if the child met the requirements for this study. A list of exclusion criteria is included in **Appendix**

4b. If the child met eligibility requirements, the child and their parent(s) were invited for a screening visit where the study was thoroughly explained to both the child and their parent(s).

During the screening visit the child and their parent(s) were provided with a recruitment letter (**Appendix 5a**) that thoroughly explained the study. Written consent was received from both the parent(s) (**Appendix 5b**) and the child (**Appendix 5c**) and any questions or concerns about the study were answered. The child was asked to repeat what they understood about the study in their own words and clarifications were provided if needed. Contact information of all researchers and study supervisors were provided on the recruitment letter and the consent forms and a copy of the consent forms were offered to the child's parent(s).

During the screening visit the following information was collected and/or verified: consumption of milk, strawberry yogurt, stringed cheese, chips, mini-sandwich type cookies and fried chips; weight; height; stage of puberty; and body fat percentage was measured using Tanita's Body Composition Analyzer (Model TBF-300A, Tanita, Arlington Heights, Illinois, USA) according to the manufacturer's instructions. Stage of puberty was assessed using the Puberty Questionnaire (**Appendix 6a**), Tanner Staging Assessment (**Appendix 6b**) and Menstrual Cycle Questionnaire (**Appendix 6c**), as appropriate. The puberty questionnaires were shown to the parent(s) first before being shown to the child if the parent(s) agreed. Parents were encouraged to assist their child with this questionnaire, if required. The children were also asked to fill out a Physical Activity Questionnaire (**Appendix 6d**) and a Dutch Eating Behaviour Questionnaire (**Appendix 6e**) that was used for secondary data analyses.

The children were asked about their preferences for their preferred type of pizza to determine which pizza choices they were served throughout the study. The Salivette® Cortisol

system (SARSTEDT AG & Co. (Nümbrecht, Germany)) was shown to the child and their parent(s) during the screening session and the child was encouraged to try chewing on the cotton ball. This was important to prevent the first-day nervousness that some children could have experienced. If the child or their parent(s) were interested in how cortisol samples were collected, the process of the saliva collection was described. After the screening session, the child was scheduled for their first session.

3.1.4 Protocol

A standardized breakfast was provided and was consumed at home on the day of each session after a fast of 12 hours and was to be consumed within 30 min. Therefore breakfast started anytime between 6:30am to 7:30am. This remained consistent throughout the duration of the study for each child. Breakfast consisted of 250mL fat-free skim milk, prepackaged 29g serving of cereal (Honey Nut Cheerios[®]; General Mills Canada Corp.) and 236mL Orange Juice (Tropicana Pure Premium[®] Orange Juice; PepsiCo Canada ULC). Children were asked to arrive at the lab any time between 9:00am and 10:00am, which remained consistent throughout the study. Children were asked to fast for 2 hours after breakfast with the exception of water, which was allowed up to one hour before arrival.

Upon arrival, children were asked to complete a questionnaire (**Appendix 7a**) about the time they slept, what time they had breakfast in the morning and whether they had consumed anything before bed the night before or after breakfast that morning. Children were also asked to complete a visual analogue scale (VAS) assessing their subjective appetite and physical comfort. VAS are 100 millimetre (mm) lines affixed with opposing descriptions at either end. Children were asked to mark an “X” on the line to depict their feelings at each time point. Scores are determined by measuring the distance (mm) from the left starting point to the intersection of the

“X”. Thus, VAS rating their motivation to eat (subjective appetite) (**Appendix 7b**) was used to describe their desire to eat (“Very weak” to “Very strong”), their feelings of hunger (“Not hungry at all” to “As hungry as I have ever felt”), their feelings of fullness (“Not full at all” to “Very full”), and how much food they could eat (“A large amount” to “Nothing at all”). After this the child completed another baseline VAS rating their physical comfort. (**Appendix 7c**) Following completion of the VAS questionnaires, the children were provided with a treatment and were asked to consume a treatment at their regular pace. The time count started at the moment when the child started to consume the treatment. The children were informed that they had 8 minutes to finish their snack. At 8 minutes the children were asked to complete VAS for the treatment sensory characteristics, including sweetness (“Not sweet at all” to “Very sweet”) (**Appendix 7d**) and pleasantness (“Not pleasant at all” to “Very pleasant”) (**Appendix 7e**) as well as another VAS rating the child’s physical comfort. VAS questionnaires for appetite and physical comfort were completed again at 15, 30, 45, 60, 90 and 120 min.

Saliva samples for cortisol assay were collected noninvasively with a synthetic cotton swab by using Salivette® Cortisol system (SARSTEDT AG & Co. (Nümbrecht, Germany) according to the manufacturer’s instructions at baseline and then at the following time points: 30, 60 and 120 min. These time points were chosen because McCarthy *et al.*(81) observed a change in salivary cortisol concentrations from right before a stressor was applied (baseline sample), and 20 minutes post-stress.

One main advantage of collecting salivary cortisol is that the method is far less invasive, and therefore less stressful, compared to serum cortisol collections. (81, 84) The use of oral stimulants is not recommended because they may affect the concentration of cortisol in the samples (95), therefore a system where the child is asked to chew on a cotton ball, such as the

Salivette® Cortisol system, (SARSTEDT AG & Co. Nümbrecht, Germany) is often used to collect salivary cortisol. (71) Another advantage of collecting salivary cortisol over serum cortisol is the bioavailability of the cortisol found in the saliva. (84) In the bloodstream, approximately 90% of the cortisol is bound to proteins and therefore only approximately 10% is considered biologically active. However, approximately 70% of the cortisol in saliva is free and are therefore able to bind onto receptors in the body. (84) This means that the level of bioavailability of the cortisol found in saliva is greater than the cortisol in blood, which in turn means that salivary cortisol is a better measurement of the amount of cortisol that is released, and therefore taken in by receptors throughout the body, during stress. Saliva samples were taken during the session where the child consumed yogurt or cookies. This was to maintain consistency with a parallel acute invasive study conducted by our research group in which short-term effects of yogurt and cookies on blood glucose control were investigated in children. A detailed description of the method of saliva collection is included in **Appendix 8**.

Ad libitum food intake was determined from a pizza meal provided at 120 min. During the meal, the children were asked to eat the pizza until they were comfortably full. Children were told that they were given approximately 30 min to eat. Two types of pizza was used for this study, McCain's Deep N' Delicious Pepperoni and/or Three Cheese Pizza (Deep N' Delicious Pepperoni or Three Cheese; McCain Foods Ltd). The reason for using this type of pizza is that the personal sized pizzas (5 inches) have no crust; therefore the toppings are spread evenly throughout the pizza. Three trays of pizza were provided for each child. Each tray held three personal sized pizzas and was served at 10 minute intervals, all children were informed of this timeline. All cooked pizzas were cut into quarters and placed randomly around each tray. This was to limit the children from keeping track of how much pizza they actually consumed. By

using the pizza ranking preferences obtained during the screening session, Children were served 2 pizzas of their top choice and 1 pizza of their second choice. In the event where a child could/would only eat one pizza choice, only their preferred pizza choice was served. Food intake (in kcal) was determined by weighing the pizza before and after serving, and converting the weight of the consumed pizza into the energy content (kcal) using the proximate nutritional analysis information provided by the manufacturer. (**Appendix 9**)

After the *Ad libitum* meal, the children were asked to fill out their last set of VAS questionnaires. During all sessions, children were supervised by the Masters student as well as by research assistants. Board games, books and a chalk board were provided to entertain and distract the children.

All children were asked to refrain from talking about food and the use of cellphones/hand held devices that may remind children of food. The children remained in a food-free area from the moment they completed the treatment up to 120 min when they moved into the feeding area where the *ad libitum* pizza meal was provided. Non-transparent screens were also used to cover the entry into the kitchen so that the children did not see the kitchen as they were moving from the non-food area to the feeding cubicles. Children sat in their own individual cubicles that prevented them from talking while eating. The use of cell phones/hand held devices was prohibited when the children were eating.

3.1.5 Ethical Considerations and Safeguards

In order to ensure the safety of the children, access to the laboratory facilities was restricted only to lab personnel. Children were constantly supervised. All lab personnel were required to provide a criminal check and a vulnerable sector search records. Lab personnel also completed WHMIS training as well as a child and lab safety orientation session. In order to

reduce the level of discomfort related to completing the Tanner Staging and puberty questionnaires that may have been uncomfortable for some children, the questionnaires were only given to the children if their parent(s) felt that the child would not be embarrassed by the questionnaire. The parent(s) were also invited to fill out the questionnaire with their child or children. A corner of the lab was sectioned off using blue screens that provided privacy for the child and their parent(s), if required.

3.2 Data Analysis

SAS version 9.2 (SAS Institute Inc, Cary, NC) was used to perform all statistical analyses. An average appetite score was calculated at each time of measurement for each treatment using the formula: $\text{Appetite Score} = [\text{desire to eat} + \text{hunger} + (100 - \text{fullness}) + \text{prospective consumption}] / 4$. (96) Therefore the average appetite was used as a summary measure of subjective appetite for statistical analyses, and a two-way repeated-measures ANOVA was used to test for treatment and time. Mean appetite scores at each time point was used to determine the total area under the curve (AUC). This provided overall mean appetite scores that was used to look at the effect of the different treatments on subjective appetite scores. Since appetite scores are not independent of each other, AUC was used because it allowed for the analysis of subjective appetite data throughout all time points and not just at a single time point. (64) It should also be noted that looking at single time points may lead to a greater degree of type 1 statistical errors because it focuses on each time point individually. (64) All other VAS scores, for example, sweetness and palatability of the treatment foods and physical comfort VAS scores were analyzed in the same way.

If there was an effect of treatment, time, and a time-by-treatment interaction on average appetite scores the Tukey-Kramer post hoc test was used to describe the mean differences

between treatments at each time point. The effects of treatment on *ad libitum* food, cortisol and on average appetite area under the curve (AUC) were determined by two-way repeated measures ANOVA followed by the Tukey-Kramer post hoc test. All ANOVAs included session as a repeated measure to control for within subject variability. If there was no effect of sex, the pooled data for boys and girls were analyzed. All results are presented as means \pm SEMs. Statistical significance was concluded with $P < 0.05$.

Salivary cortisol samples were analyzed using a commercial Salivary Cortisol ELISA kit by Eagle Biosciences (Eagle Biosciences Inc. Boston, Massachusetts, USA) at the IWK research laboratory (PI: Dr Y. Anini). See **Appendix 10** for a summary of the ELISA procedure.

Chapter 4 Results

4.1 Subject Characteristics

Twenty seven children were recruited for this study. One participant was dropped from the study due to bad behaviour during the study sessions that were distracting for the other participants. Another participant was dropped from the study due to loss of follow up. One participant failed to pass the telephone screening questionnaire and another participant would have been too old to include in this study upon completion of all five sessions. In total, twenty three normal weight children (16 girls and 7 boys in the 5th-85th BMI percentile for age and gender) participated in this study. The mean age was 11.55 ± 0.27 years, with a mean BMI percentile of 45.7 ± 5.6 . Baseline characteristics for all participants, including Dutch Eating Behavioural Questionnaire (DEBQ) scores and Body Fat %, are listed in Table 1.

Table 1: Subject Characteristics

Age (years)	11.55 ± 0.27
Weight(kg)	39.38 ± 2.6
Height (m)	1.47 ± 0.02
Physical Activity Level (METs) per week	35.86 ± 5.75
BMI (kg/m ²)	17.7 ± 0.47
BMI %ile	45.7 ± 5.62
FM (kg)	6.61 ± 0.89
FM (%)	16.36 ± 1.52
Tanner Staging	1.35 ± 0.24
DEBQ Average Score	1.51 ± 0.06
DEBQ Restraint	1.38 ± 0.09
DEBQ Emotional	1.17 ± 0.06
DEBQ External	2.03 ± 0.10

Data are reported as means ± SEM, n = 23. Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat-free mass; DEBQ, Dutch Eating Behaviour Questionnaire

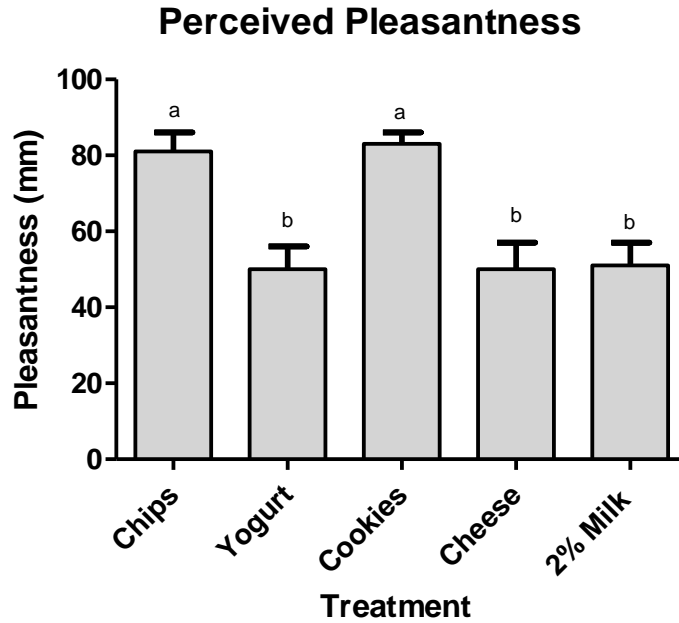
4.2 Sensory Characteristics of the Treatments

Five isocaloric (180kcal) treatments were used in the study:

- (a) 2% M.F. milk, 346.2g
- (b) Greek Yogurt, 0% M.F. with strawberry, 200g
- (c) Mozzarella cheese, 63g
- (d) Mini sandwich type cookies, 38.6g
- (e) Potato chips, 32.1g

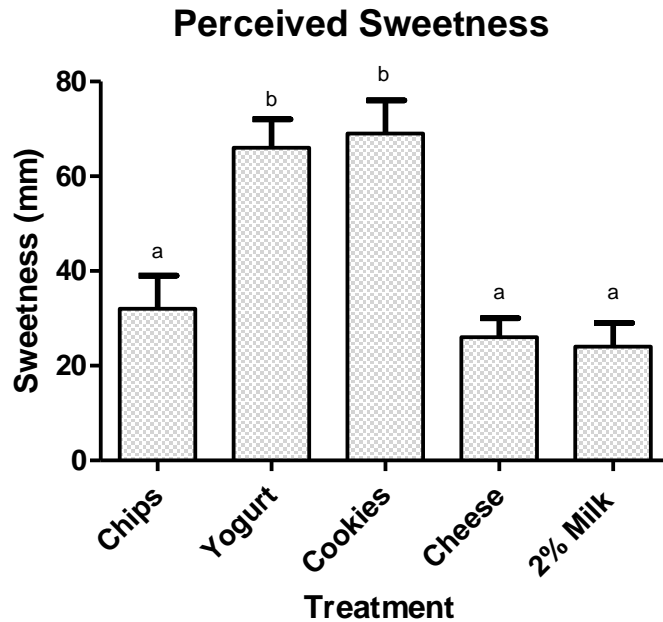
There was an effect of treatment on perceived pleasantness and sweetness. ($P < 0.05$) It was found that the chips and cookies resulted in significantly higher subjective pleasantness compared to yogurt, cheese and milk (49.8 ± 6.3 , 50.5 ± 6.9 and 51.0 ± 6.2 mm, respectively). ($P < 0.01$) (Figure 1) The participants found that the yogurt and cookies (66.3 ± 6.4 and 68.8 ± 7.1 mm, respectively) were significantly sweeter than chips, cheese and milk (31.7 ± 7.5 , 26.3 ± 3.9 , 24.3 ± 5.4 mm, respectively). ($P < 0.05$) (Figure 2)

Figure 1: Treatment Pleasantness



Two-way ANOVA with a Tukey Kramer Post-hoc test. Mean \pm SEM, n=23. Values with different superscripts are significantly different. (P<0.05)

Figure 2: Treatment Sweetness

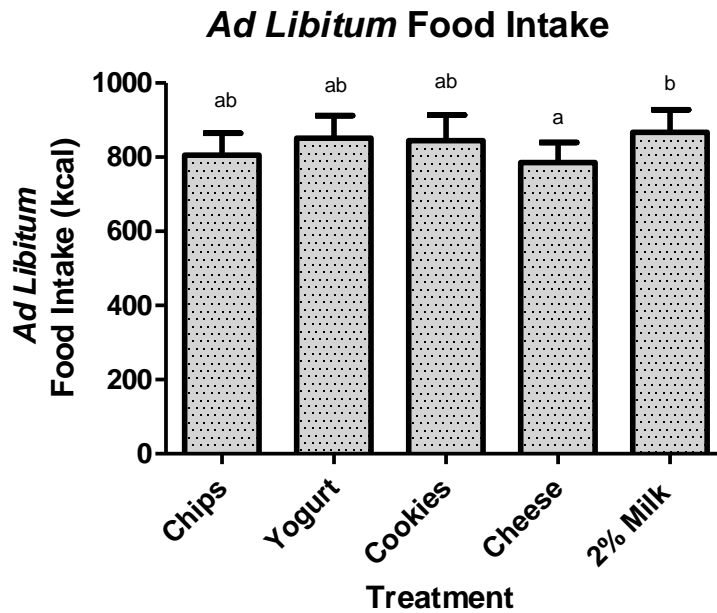


Two-way ANOVA with a Tukey Kramer Post-hoc test. Mean \pm SEM, n=23. Values with different superscripts are significantly different. (P<0.05)

4.3 Food Intake

Ad Libitum food intake (in kcal) was affected by treatment, ($P=0.0258$) (Figure 3) (Table 2) but not by session ($P=0.1125$) or sex ($P=0.2227$). There were no significant interactions. The treatments with milk and cheese resulted in the highest and lowest *ad libitum* food intake, respectively and were significantly different ($p<0.05$) while other treatments resulted in intermediate intake. (Figure 3)

Figure 3: *Ad Libitum* Food Intake

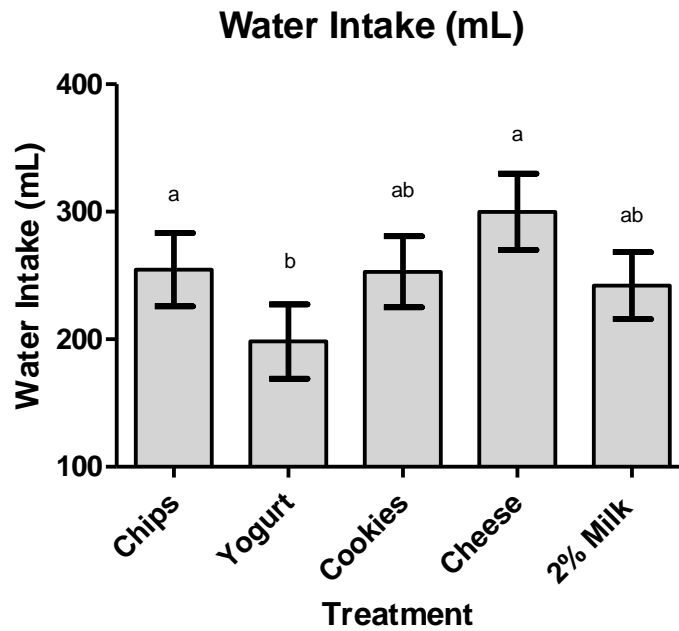


Two-way ANOVA with a Tukey Kramer Post-hoc test. Mean \pm SEM, $n=23$. Values with different superscripts are significantly different. ($P<0.05$)

4.4 Water Intake

Water intake (in mL) was affected by treatment ($P=0.0009$) (Figure 4), but not by session ($P=0.1760$) or sex ($P=0.5456$). There were no significant treatment-by-session interactions ($P=0.1790$). These results are also displayed in Table 2. The highest water intake was observed after the treatment with cheese (230.0 ± 30.0 mL) and the lowest after the treatment with yogurt (198.3 ± 29.1 mL).

Figure 4: Water Intake

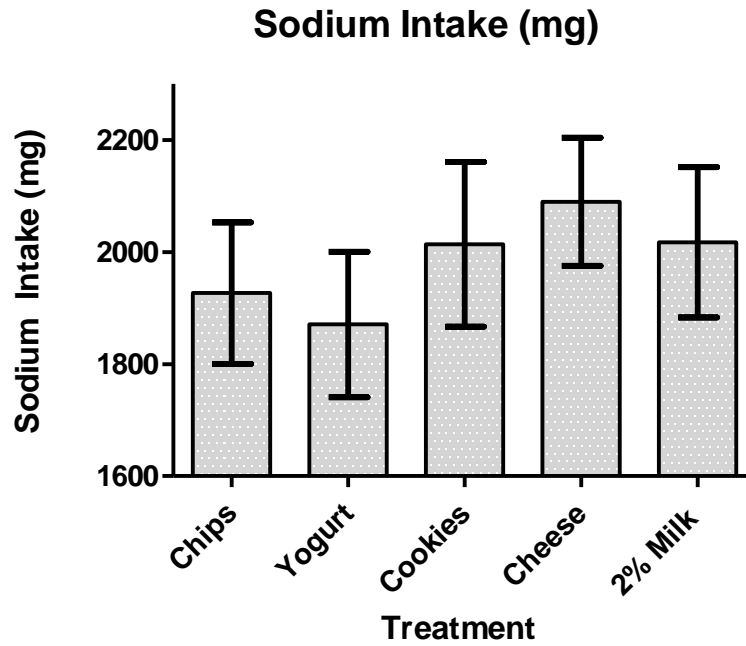


Two-way ANOVA with a Tukey Kramer Post-hoc test. Mean \pm SEM, n=23. Values with different superscripts are significantly different. (P<0.05)

4.5 Sodium Intake

Sodium intake with the treatment and *ad libitum* meal was not affected by treatment (P=0.0641) (Figure 5), sex (P=0.0922) or session (P=0.0596). There were no significant treatment-by-session interactions (P=0.5523). These results are displayed in Table 2.

Figure 5: Sodium Intake



Two-way ANOVA. Mean ± SEM, n=23.

Table 2: Effect of Treatments on Food Intake, Water Intake, Sodium Intake, Treatment Pleasantness and Sweetness, and Physical Comfort

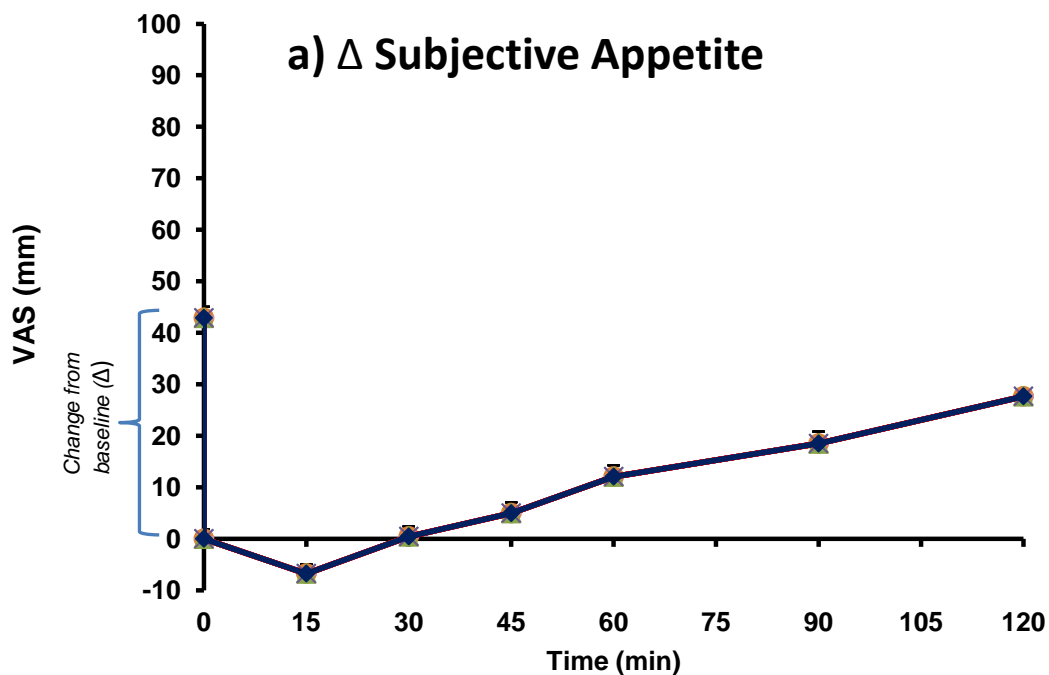
	Chips	Yogurt	Cookies	Cheese	2% Milk
<i>Ad libitum</i> Food Intake (kcal)	796 ± 61 ^{ab}	851 ± 61 ^{ab}	845 ± 69 ^{ab}	785 ± 54 ^a	867 ± 61 ^b
Water Intake (mL)	255 ± 29 ^a	198 ± 29 ^b	253 ± 28 ^{ab}	300 ± 30 ^a	242 ± 26 ^{ab}
Sodium Intake (mg)	1927 ± 126	1871 ± 130	2014 ± 147	2090 ± 114	2017 ± 134
Pleasantness (mm)	81 ± 5 ^a	50 ± 6 ^b	83 ± 3 ^a	50 ± 7 ^b	51 ± 6 ^b
Sweetness (mm)	32 ± 7 ^b	66 ± 6 ^a	69 ± 7 ^a	26 ± 4 ^b	24 ± 5 ^b
Physical Comfort (mm)	78 ± 1	68 ± 2	77 ± 2	70 ± 2	71 ± 2

Data analyzed using two-way ANOVA with a Tukey Kramer Post-hoc test. Mean ± SEM, n=23. Values with different superscripts are significantly different. (P<0.05)

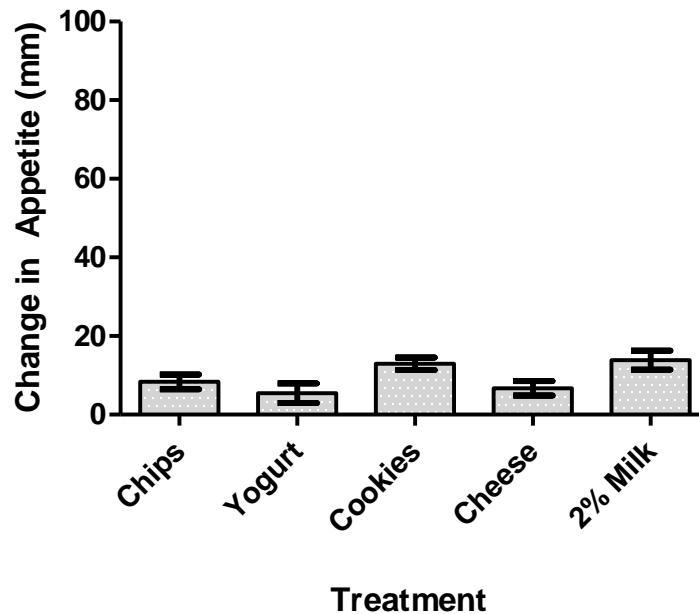
4.6 Subjective Appetite Scores

There was an effect of treatment on the average appetite score at baseline ($P < 0.05$), therefore the change from baseline values (Δ) were used for the calculation. The subjective appetite scores for the period from 0 to 120 min were affected by time ($P < 0.0001$) (Figure 6a), but not by treatment ($P = 0.2$) (Figure 6b), session ($P = 0.2$) or sex ($P = 0.0705$). The subjective appetite scores increased as time increased, regardless of treatment. There was no effect of treatment, ($P = 0.1226$) session ($P = 0.3614$) and sex ($P = 0.4687$) on post-meal appetite at 145 min.

Figure 6: Subjective Appetite



b) Cumulative (2 hours) Δ Subjective Appetite Scores



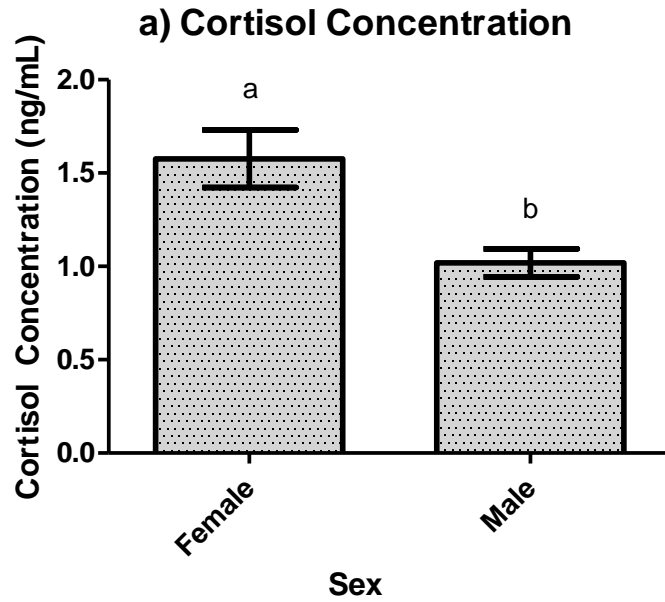
Two-way ANOVA. Mean \pm SEM.

4.7 Cortisol

Cortisol concentration was affected by age ($P=0.0004$), sex ($P=0.0075$) (Figure 11a), but not by treatment ($P=0.9198$), time ($P=0.0979$) (Figure 11b) or session ($P=0.2209$). There was a significant age-by-sex interaction ($P=0.0004$), treatment-by-sex interaction ($P<0.0001$) and sex-by-session interaction ($P=0.0002$).

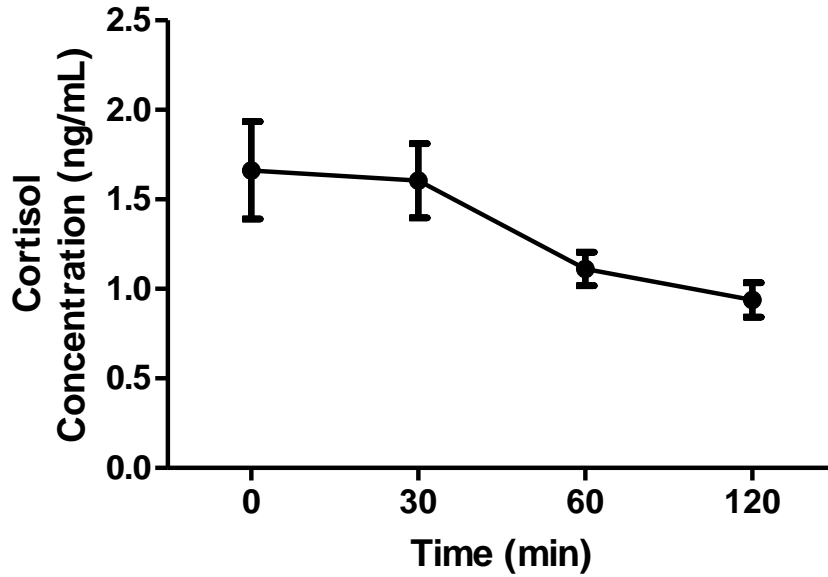
Total area under the curve for cortisol concentration was not affected by treatment ($P=0.9868$) (Figure 11c), session ($P=0.1287$) (Figure 11d) or sex ($P=0.0822$). There were no significant interactions.

Figure 7: Cortisol



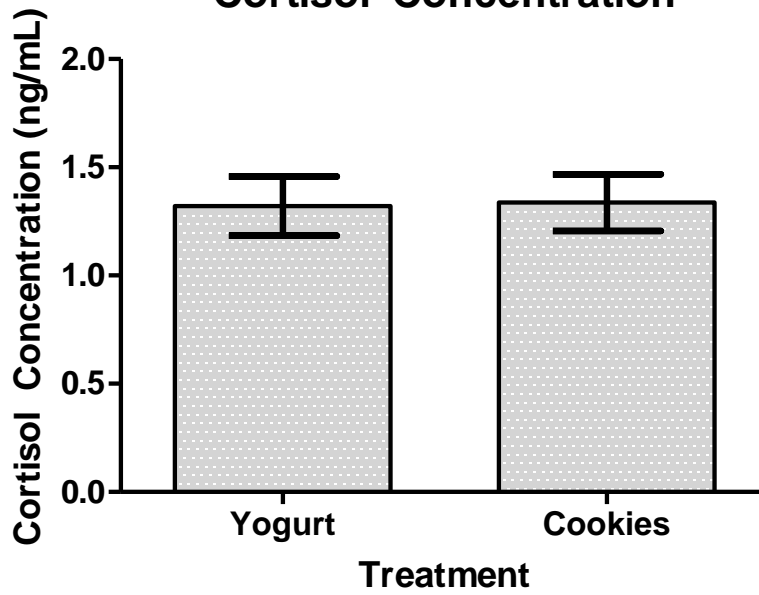
Two-way ANOVA with a Tukey Kramer Post-hoc test, represented as Mean \pm SEM, n=18. Values with different subscripts are significantly different. (P<0.05)

b) Cortisol Concentration

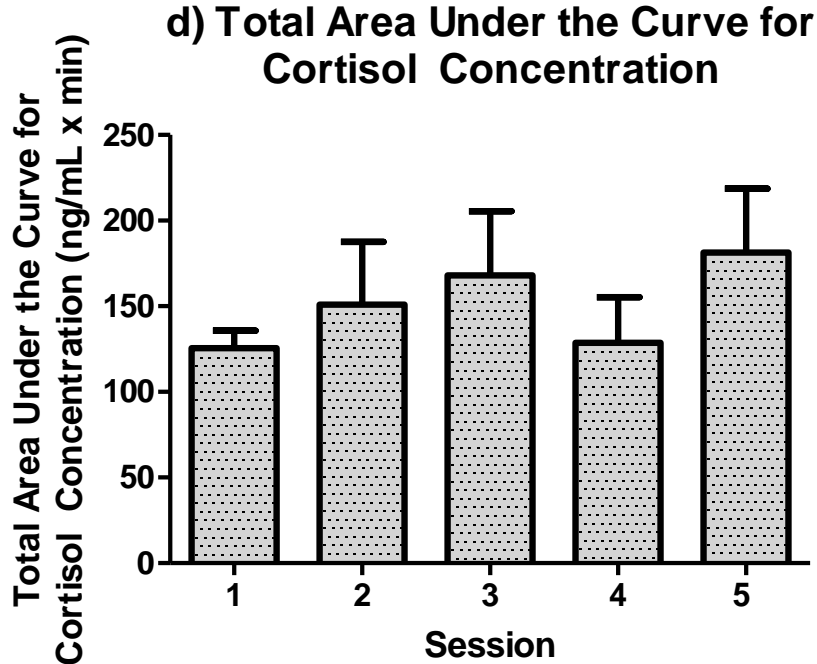


Two-way ANOVA. Mean \pm SEM, n=18.

c) Total Area Under the Curve for Cortisol Concentration



Two-way ANOVA. Mean \pm SEM, n=18.



Two-way ANOVA. Mean \pm SEM, n=18.

4.8 Correlations

4.8.1 Subject Characteristics

BMI was positively correlated to FM percent, $r=0.70$ ($P=0.0004$) and restrictive eating, $r=0.52$ ($P=0.0117$). Percent body fat was positively correlated to restrictive eating, $r=0.53$ ($P=0.0139$). Overall DEBQ was positively correlated to Tanner Staging, $r=0.44910$ ($P=0.0316$).

4.8.2 Food Intake

Ad libitum Food intake following all treatments was not correlated to: Tanner staging, BMI, Physical Activity level, %FM or overall DEBQ scores or appetite scores. (Table 3) Food intake was positively correlated to age following: chips, $r=0.45492$ ($P=0.0292$); cookies, $r=0.49332$ ($P=0.0168$); and 2% milk, $r=0.54745$ ($P=0.0069$) (Table 3). Treatment weight (in g) was positively correlated to *ad libitum* food intake following: chips, $r=0.59401$ ($P=0.0028$); yogurt, $r=0.56909$ ($P=0.0046$); cookies, $r=0.63493$ ($P=0.0011$); and cheese, $r=0.62825$

(P=0.0013). *Ad libitum* food intake following all treatments was not correlated to water consumption, treatment sweetness, treatment pleasantness or physical comfort, but was positively correlated to sodium consumption following all treatments (Table 3). Water intake following all treatments (in mL) was not correlated to sodium intake (in mg).

Table 3: Food Intake Correlations

	FI Chips	FI Yogurt	FI Cookies	FI Cheese	FI 2% Milk
Age	r = 0.45492*	r=-0.27656	r=-0.49332*	r=0.29765	r=0.54745*
Tanner Staging	r= 0.10382	r= -0.08532	r=0.10457	r=0.15108	
BMI	r= 0.29918	r= 0.27273	r= 0.26191	r= 0.23217	r= 0.29050
% FM	r= 0.28167	r= 0.33513	r= 0.39979	r= 0.37864	r= 0.17535
Physical Activity Level (METs)	r= 0.23927	r= 0.02052	r= 0.04408	r= 0.20056	r= 0.08809
Overall DEBQ	r= -0.02727	r= -0.10008	r= -0.09541	r= -0.12882	r= -0.19275
Treatment Weight (g)	r= 0.59401*	r=-0.56909*	r=0.63493*	r=0.62825*	r=0.36252
Absolute Appetite Scores (mm)	r =-0.06028	r=0.28143	r=0.03188	r=-0.04353	r=-0.18584
Sodium Intake (mg)	r= 0.99437*	r= 0.99443*	r= 0.99591*	r= 0.99370*	r= 0.99487*
Water Intake (mL)	r=0.03234	r=0.12919	r=0.22292	r= 0.08863	r= 0.25934
Sweetness (mm)	r=0.28100	r=-0.10845	r=-0.17737	r=0.48481	r=-0.08729
Pleasantness (mm)	r=-0.05035	r=0.16387	r=-0.08412	r=0.41350	r=0.25287
Physical Comfort (mm)	r=-0.15402	r=0.13152	r=0.09654	r=0.07736	r=-0.09482

*Pearson Correlation Coefficients, *Indicates P<0.05.*

4.8.3 Cortisol

Cortisol concentration following the yogurt and cookie treatments was not correlated to BMI, age, FM%, physical activity, Tanner staging, overall DEBQ, physical comfort, pizza intake, cumulative food intake, subjective appetite scores or treatment pleasantness or sweetness. (Table 4) Cortisol concentration following the cookie treatment was not correlated to total AUC, however, cortisol concentration following the yogurt treatment was positively correlated to total AUC (P=0.0104). (Table 4)

Table 4: Cortisol Concentration Correlations

	Cortisol Concentration following Yogurt Treatment	Cortisol Concentration following Cookie Treatment
Age	r=0.12343	r=-0.22335
Tanner Staging	r=0.19057	r=-0.16232
BMI	r= -0.12220	r= 0.30181
% FM	r=-0.14030	r= 0.07786
Physical Activity Level (METs)	r= -0.32487	r=-0.31390
Overall DEBQ	r=-0.17651	r=-0.04955
Absolute Appetite Scores (mm)	r=-0.21159	r= 0.35249
Desire to Eat Scores (mm)	r=-0.21489	r= 0.21615
Hunger Scores (mm)	r= -0.17823	r= 0.21433
Fullness Scores (mm)	r= 0.28292	r=-0.28160
Perceived Food Consumption Scores (mm)	r=-0.09613	r=0.45225
Total AUC for Absolute Appetite Scores (mm x min)	r= 0.58719*	r=0.42662
Physical Comfort (mm)	r= 0.09381	r= -0.31647
Pizza Intake (kcal)	r=-0.41314	r= 0.05267
Cumulative Food Intake (kcal)	r= -0.41314	r=0.05267
Sweetness (mm)	r= 0.16352	r=-0.22427
Pleasantness (mm)	r= -0.14932	r= -0.29779

*Pearson Correlation Coefficients, *Indicates P<0.05.*

Chapter 5 Discussion

This study supports the proposed hypothesis and demonstrates that certain dairy products have a strong potential to suppress *ad libitum* food intake in children. The magnitude of the effect of dairy products on subsequent food intake is predetermined by various factors including physical and chemical characteristics such as an aggregate state and the macronutrient composition, namely protein content. In the present study, *ad libitum* food intake was 82 kcal lower following the cheese treatment compared to the treatment with 2% milk (785.0±54.4 and 867.1±60.8 kcal, respectively, P<0.05). Cheese was the only solid dairy product used in this

study while two other non-dairy solid products were potato chips and cookies; however, there were no statistically significant differences between these non-dairy solid treatments and milk. This suggests that two factors are important in suppressing subsequent food intake: dairy matrix and solid physical state. None of these factors alone have the same effect. For instance, both dairy products, cheese and yogurt, had a high protein content (18 and 17 g per serving of 180 kcal, respectively) but only the cheese treatment resulted in significantly lower food intake (-82 kcal) while the food intake after the treatment with yogurt was similar to that after the treatment with milk (851.2 ± 60.7 and 867.1 ± 60.8 kcal, respectively). Thus the difference in food intake after the treatments with cheese and yogurt was 66 kcal ($P=0.067$). Similarly, a solid state of a food *per se* does not predict its effect on subsequent food intake. Both non-dairy solid treatments led to intermediate intake (796.4 ± 61.4 and 844.9 ± 68.9 kcal after chips and cookies, respectively) that was not significantly different from food intake after the milk treatment (867.1 ± 60.8 kcal). One of the most distinctive characteristics of dairy matrix is high content of protein. Thus the protein content of the treatment with cheese was 18 g while the treatments with cookies had only 1.3 and 1.9 g of protein, respectively. Both treatments with cheese and chips were solid and had similar content of fat (12 and 11.6 g, respectively), but the combination of a solid matrix and high fat content in fried potato chips did not result in the same synergistic effect as it was observed for a solid state and high protein content immanent to cheese. The earlier study of Anderson & Moore showed that among the macronutrients, protein provides the highest level of satiety. (66) However this effect is greatly determined by the origin of the protein. In general, both major milk protein fractions are known for their intake-suppressing properties. Thus it was shown that whey protein was better at reducing short-term food intake compared to soy and egg proteins. (67) Whey protein is also known for many other metabolic benefits and is widely

consumed as a supplement. (97) While whey is known to exert physiological effects shortly after ingestion, casein is more satiating in the long-term. (98) Mozzarella cheese used in this study contains predominantly coagulated casein. While it is known that whey protein and casein exhibits different kinetics of digestion and absorption in gastrointestinal tract (69), the extended digestion distinctive for casein can be enhanced by a solid matrix that requires more time for hydration compared to fluid. (99) This can explain the effect of cheese on subsequent food intake in two hours as synergism of milk protein and solid physical state.

Several studies have explored the possible relationship between food texture and perceived satiety and food intake. (100-106) It is hypothesized that liquids are less satiating than solid foods and therefore promote energy intake. (100-102, 104-106) This idea comes from the fact that absorption in the distal portion of the GIT can only happen after gastric emptying starts. (104) There are also stretch receptors within the walls of the stomach that send signals to the brain when it is full that helps promote the ending of a meal. (107-110) Therefore the rate at which food is emptied from the stomach, into the duodenum may determine how satiating a food product is. (107) Using MRI scanning technology, Marciani, *et al.* found that meal viscosity significantly delayed gastric emptying and that there was a positive relationship between meal viscosity and the sense of fullness. (107) Another idea to support this hypothesis is related to oral processing time. (111) The more time a food product spends in the mouth, the greater the exposure one has to the smell, taste and mouth feel of the food product. (111) Zijlstra, *et al.* confirmed this when they found that there was an inverse relationship between oral processing time and bite size food intake. (111) It has been hypothesized that the act of chewing may send signals to the brain in preparation for the consumption of food, and therefore a solid food may provide greater satiety than a liquid food product. (100)

Liquid food products have been shown to be less satiating than solid (101-103, 112) or semi-solid (100, 113) food products, but rarely does this translate to food intake. A study looking at the effect of food viscosity of a chocolate flavoured drink on *ad libitum* food intake found that participants consumed significantly more liquid product compared to the semi-liquid product and the semi-solid product. (100) All food products were isocaloric and equal in macronutrients and therefore the differences seen in this study could not have been due to caloric or nutritional differences. (100) Chapelot and Payen conducted a study that compared the effects of solid chocolate and liquid yogurt consumed in the afternoon on satiety. (112) The participants in the study were first provided with a fixed lunch (approximately 670kcal) then 4 hours later were provided with either the solid or liquid preload, which were matched for density, volume (366mL) and energy content (284kcal). (112) They found that regardless of preload, there was no difference in the amount of time between the consumption of the preload and the request for dinner. (112) Contrary to other studies, it was found that subjective appetite was stronger in the liquid yogurt, compared to the chocolate bars, (112) which could be related to the differences in macronutrients of the two preloads. However, this difference in subjective appetite did not translate to *ad libitum* food intake at a meal served at the participant's request, which was in general served 170min post preload consumption. (112) Similarly in this study, all treatment resulted in similar appetite suppression, however only the treatment with cheese resulted in lower food intake compared to milk which indicate that the changes in appetite might not precisely predict the following food intake.

Almiron-Roig, *et al.* conducted a study where a solid (cookies) or liquid (regular cola) preload was provided either 20min or 2 hours before an *ad libitum* test meal to test the effect of satiety and food intake. (105) It was found that there was no difference in subjective hunger and

desire to eat scores between the two preloads. However, it was interesting to see that the liquid preload provided greater feelings of fullness immediately after consumption, compared to the solid preload. (105) At the test meal, it was found that there was no difference in the amount consumed when comparing the liquid and the solid preload, but that there was a difference depending on the time delay of the test meal. (105) It was found that when preloads were served 20 minutes before the test meal, the participants consumed significantly less than when the preload was served 2 hours before the test meal. (105) This suggests that the time between the preload and the test meal may be a factor in energy intake. It should be noted that the cookies provided energy from both carbohydrates and proteins and included fibre, whereas the calories from the regular soda was from carbohydrates alone. (105)

In order to simulate a real-life situation, as suggested by Blundell *et al.* (64), it was important that the timing between the treatment and *ad libitum* food intake as well as the caloric content of the treatments in this study were planned at 120min and 180kcal, respectively. In general, a healthy, nutritious snack should be one that is approximately 140kcal. Also, 2 hours after the consumption of a meal is a time where most children in school have the opportunity to consume a snack, for example during recess or after school. Since the treatments were matched for caloric content, the weights of the treatments were all different. Therefore it was interesting to see that there was only a difference in *ad libitum* food intake between the cheese and 2% milk treatments because the weight of the treatments ranged from 32.1g (potato chips) to 346.2g (2% milk).

There is strong evidence to support the hypothesis that gastric distention promotes satiety. (109, 114) The idea is that vagus nerves in the stomach will send signals to the brain, namely to the amygdala and the insular cortex, to promote satiety when stomach receptors are

stretched. (109) Although the difference in weight and volume between the treatments with cheese and milk is more than 5 times, the higher weight and volume of the milk treatment (346 g) is not a factor in determining the lower subsequent food intake compared to the treatment with cheese (63 g). There was also a positive correlation observed in the present study between the weight of all solid (chips, cookies, cheese) and semi-solid (yogurt) treatments and *ad libitum* food intake ($r=0.59401$; $r=0.63493$; $r=0.62825$; $r=0.56909$, respectively, $P<0.05$), whereas the weight of the liquid milk treatment, with the greatest weight, was not correlated to *ad libitum* food intake. ($r=0.36252$, $P>0.05$) This further confirms the theory that both dairy matrix and a solid physical state are two important factors in the suppression of subsequent *ad libitum* food intake. It was also interesting to see that even though there was a difference in the volume of water consumed during the *ad libitum* meal, and therefore there was a difference in stomach distension, there was no correlation between water consumption and *ad libitum* food intake.

Visual Analogue Scales (VAS) are often used to determine subjective appetite, physical comfort and treatment characteristics (115, 116), however there is a concern whether children can understand the concept of VAS and correctly indicate their feeling. (117) In the present study, the participants were able to correctly determine that the cookies were significantly sweeter than yogurt, 2% milk, chips and cheese, which provides evidence to support that the participants in the study understood how to properly fill out the VAS questionnaires. The participants preferred the chip and cookie treatments over the dairy treatments, which is not surprising seeing that the top after-school snack preference in Canadian children are cookies, (51) and the top snack choice in America is chips. (53) This observation also falls in line with the current decline in the consumption of dairy in children. (56-58) Another interesting finding regarding the treatment characteristics was that there was no correlation between pleasantness,

sweetness or physical comfort and *ad libitum* food intake, and therefore it can be concluded that treatment characteristics were not the cause of the difference seen in *ad libitum* food intake.

The significant differences in the consumption of water between the treatments in the present study were not surprising. The delicate balance between sodium and water concentrations in the human body are tightly regulated by the hypothalamus in that the body will usually respond to an elevated sodium concentration by increasing thirst. (21) According to the nutrition labels provided by the manufacturers and by Maxxam Analytics, the amount of sodium from the least to greatest was yogurt, 2% milk, chips, cookies and cheese, and the highest water intake was observed after the cheese treatment.

There have been many studies that have explored the possible relationship between dairy intake and satiety. (105, 118-127) This is mainly due to the satiating properties of the macronutrients within milk, for example whey and casein. (65, 67, 128, 129). In the present study, it was found that the change in appetite scores was only affected by time. Generally, as time passes, appetite will usually increase as well. Therefore, it was not surprising then that change from baseline appetite scores increased as time went on, regardless of treatment. This may also suggest that children are able to assess their subjective appetite sensations using VAS as there were some concerns that VAS is not a reliable instrument to assess food intake in schoolchildren. (117)

A study by Hollis, *et al.* explored whether dairy consumption would influence food intake and found that there were no differences in subjective appetite scores and instead found that participants consumed more calories during the high-dairy consumption period than during the low-dairy consumption period. (121) The participants were separated into a high-dairy

condition (three portions of dairy per day) or a low-dairy condition (one portion of dairy per day) for 7 days, however, it should be noted that the participants recruited for this study were all adults who were overweight or obese (BMI between 25-32kg/m²) (121), and it is hypothesized that the hormonal control of appetite in this population is compromised. (15, 17, 21) In another study by Oritinau, *et al.* comparing a high-protein Greek yogurt to a regular yogurt found that there was no difference in perceived hunger, or fullness or *ad libitum* food intake among the two treatments. (120) It should be noted that similar to the present study, the calorie content of the treatments in this study was 160kcal, as mentioned above, a calorie content of at least 200kcal is needed before caloric compensation is seen at an *ad libitum* meal 60 min later. (127, 129, 130)

While the above studies did not see a difference in subjective appetite, it should be noted that there have also been studies that have found a difference in subjective satiety in dairy products. In a study comparing isoenergetic (kcal) and isovolumetric amounts of milk, cheese, yogurt and water (control) it was found that hunger and desire to eat scores were the lowest following yogurt compared to cheese, milk and the control. (122) Using yogurt with low, moderate and high protein content, Douglas, *et al.* assessed subjective appetite and *ad libitum* food intake. (131) The treatments were isocaloric (160kcal) and had 5g, 14g or 24g of protein and the *ad libitum* test meal was served upon participant's request. It was found that the high protein yogurt had suppressed appetite, compared to both moderate and low protein yogurt. There was no difference between the moderate and the low protein yogurts in subjective appetite. (131) Other studies have also found that protein contents less than 23g/250mL are required in order to see an effect on food intake and subjective appetite. (129) However in the present study, the treatment with cheese had 18 g of protein which is less than in the study cited above, which may explain why there was no difference in subjective satiety in the present study.

In the present study, the baseline cortisol concentrations for girls and boys ($1.6 \pm 0.15\text{ng/L}$ and $1.02 \pm 0.07\text{ng/mL}$, respectively) were different, compared to those of another study. In a study exploring morning cortisol concentrations in Swedish children aged 7-15 years old, it was found that both girls and boys had a similar mean cortisol concentration of 8.6 nmol/L (3.12ng/mL) and 8.8 nmol/L (3.19ng/L), respectively. (80) It should be noted that this study took samples from 8-9 in the morning, in the child's classroom and there was no control over the child's regular morning routine before arriving to school. However, when explored via age cohort there was a difference in cortisol concentration depending on the age of the child. Comparing the general pattern of the results from the present study and the results from Tornhage (80), it can be concluded that the results from the present study fall within the lower range that was seen in the study by Tornhage, as well as within the range seen in a descriptive study described earlier by McCarthy, *et al.* (81)

The results from the present study also show a similar pattern seen in the study by Tornhage in that those who were 10 and 14 years old had the lowest cortisol concentration, which those who were 12 years old had the highest cortisol concentration. (80) This difference in cortisol concentration can be explained by the stages of puberty as most participants in this age group were in the mid- to post-pubertal stages. There is only one study that looked at the cortisol response to food intake in children, which was conducted by Hershberger *et al.* This study showed that cortisol concentration in normal weight children increased in response to lunch, (39) however, this was not seen in the present study. Instead it was found that the mean cortisol concentration in the present study decreased as time went on. This is likely due to timing, as the participants in the study by Hershberger *et al.* (39) consumed breakfast at 10am and lunch at 2pm and were also subjected to 30 minutes of exercise 1 hour before lunch, while the

participants in the present study consumed breakfast at 6:30-7am, consumed a snack around 9:30-10am and consumed lunch at 11:30am or 12:00pm. The circadian rhythm of cortisol should also be considered when reviewing these results. Therefore, if looking at the general time pattern of the cortisol concentrations, there was a dip in cortisol concentration around noon in the present study, the study by Hershberger *et al.* (39) and in the study by McCarthy, *et al.* (81)

To summarize, this study provides the important information on popular snack products and their effects on food intake regulation in children. There are some limitations, future directions and implications that are addressed below.

Chapter 6 Limitations

One major limitation of this study was that there was a treatment effect seen at baseline for average appetite. One reason that may have contributed to this may have been related to poor randomization. This may explain the session effect on *ad libitum* food intake (in g) and the treatment effect at baseline seen in the present study. Another potential reason for the difference seen at baseline may have been related to participant compliance. Upon arrival to the lab, all participants were asked to fill out a questionnaire that ensured that the protocol procedures were followed, also all participants were reminded that breakfast was to be consumed in full 2 hours prior to their arrival at the lab, however, there is no way to ensure that these procedures were followed, other than the questionnaire. Another reason for this may have been that participants did not fully understand how to complete the VAS questionnaires. However, since there was a positive effect of time on change from baseline subjective appetite scores ($P < 0.0001$) and participants were able to correctly identify the saltiness of the treatments, it may be safe to say that participants understood how to properly fill out the VAS questionnaires.

Another potential limitation of this study was the select study population as only children within the Halifax Regional Municipality (HRM) were included in this study. As mentioned earlier, a study found that the children living in the Atlantic Provinces in 1996 were 1.45 times more likely to be overweight or obese compared to the rest of Canada. (12) Therefore, the results of this study may only be reflective of the children within the HRM or within Atlantic Canada, however, there was a similar study that was conducted in Toronto, Ontario which may shed light on the food intake patterns of children in Toronto as well. The disproportionate number of girls and boys may be another limitation. It is known that girls and boys enter puberty at different times in that girls generally enter puberty earlier than boys. There have also been several adult studies that have found differences amongst males and females with respect to food intake and subjective appetite. (132-134)

Puberty is a time of growth and development that is extremely hard to control. Since some of the participants in this study were going through puberty, their appetite and hormones might have been affected. While this was a major concern, it should be noted that less research in this age group is available for this very reason, and therefore a small study that looks at short-term effects of food intake on subjective satiety and energy intake at a test meal was able to shed some light on this population and maybe even encourage the inclusion of children who may be going through puberty.

While pictures and conversations about food were prohibited during all study sessions, there is no way to completely avoid it. Since anticipation of food intake plays a role in appetite, it was hard to control this aspect of appetite. For this reason, there were various board games and books that were used to help distract the participants as well as various volunteers who were able to help ensure that there were no conversations about food or appetite. The participants also

consumed all food products in individual cubicles which helped control the social aspect of food consumption and allowed the participants to focus on their internal cues.

Chapter 7 Conclusion

A solid dairy snack (Mozzarella cheese) is effective in reducing food intake in children. Yogurt and milk suppresses food intake similarly to other popular non-dairy snacks, however, they provide better nutrient profiles.

Chapter 8 Implications of the Study and Future Directions

This study provides valuable information that may help develop new guidelines in the prevention and treatment of obesity as more research is needed to determine new methods to prevent or treat obesity. This study provides the evidence to promote cheese as the healthy snack rich in nutrients and capable to reduce food intake. Similarly milk and yogurt can be positioned as snack products that can affect food intake similarly to cookies and chips, but contrary to these two popular snacks, milk and yogurt have better and healthier nutrient profiles.

Dairy products contain several vitamins and minerals that are essential for growth and development in this population; however, there has been a decline in the consumption of dairy products in Canadians, especially Canadian children. (56) This study provides support for the need to encourage children and youth to consume dairy products more often over traditional snack items such as cookies and chips as a snack. As suggested by Raj, *et al.* (36) it is essential that guidelines and policies for weight loss/maintenance emphasize the development of a healthy eating habits, especially in children.

References

1. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Pediatr Obes* 2006;1(1):11-25.
2. Ekmekcioglu C, Touitou Y. Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. *Obes Rev* 2011;12(1):14-25.
3. Tremblay MS, Katzmarzyk PT, Willms JD. Temporal trends in overweight and obesity in Canada, 1981-1996. *Int J Obes* 2002;26(4):538-543.
4. Guo SS, Wu W, Chumlea WC, Roche AF. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr* 2002;76(3):653-658.
5. Ogden CL, Flegal KM. Changes in Terminology for Childhood Overweight and Obesity. In: Services USDoHaH, editor.; 2010.
6. Veugelers PJ. Prevalence of and risk factors for childhood overweight and obesity. *Can Med Assoc J* 2005;173(6):607-613.
7. Sallis JF, Glanz K. The Role of Built Environments in Physical Activity, Eating, and Obesity in Childhood. *Future Child* 2006;16(1):89-108.
8. Tremblay MS, Willms JD. Secular trends in the body mass index of Canadian Children *Can Med Assoc J* 2000;163(11):1429-1433.
9. Malina RM, Katzmarzyk PT. Validity of the body mass index as an indicator of the risk and presence of overweight in adolescents. *Am J Clin Nutr* 1999;70 (Supplement):131S-136S.
10. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The Relation of Childhood BMI to Adult Adiposity: The Bogalusa Heart Study. *Pediatrics* 2005;115(1):22-27.
11. Katzmarzyk PT, Ardern CI. Overweight and Obesity Mortality Trends in Canada, 1985-2000. *C J Public Health* 2004;95(1):16-20.
12. Willma JD, Tremblay MS, Katzmarzyk PT. Geographic and Demographic Variation in the Prevalance of Overweight Canadian Children. *Obes Res* 2003;11(5):668-673.
13. Shields M. Overweight and obesity among children and youth. *Health Rep* 2006;17(3):27-42.
14. Hellström PM, Geliebter A, Näslund E, Schmidt PT, Yahav EK, Hashim SA, et al. Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *British Journal of Nutrition* 2007;92(S1):S47.
15. Stanley S, Wynne K, McGowan B, Bloom S. Hormonal Regulation of Food Intake. *Physiol Rev* 2005;85(4):1131-1158.
16. Akhavan T, Panahi S., Anderson G.H., B.L. L. Application of dairy-derived ingredients in food intake and metabolic regulation. In: Application of dairy-derived ingredients in food intake and metabolic regulation. Cambridge, UK: Woodhead Publishing Ltd.; 2009. p. 212-237.
17. Hillebrand JG, de Wied D, Adan RAH. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides* 2002;23(12):2283-2306.
18. Stellar E. The Physiology of Motivation. *Psychol. Rev.* 1954;61(1):5-22.
19. Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, et al. Leptin Activates Hypothalamic CART Neurons Projecting to the Spinal Cord. *Neuron* 1998;21(6):375-1385.
20. Pellemounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the *obese* Gene Product on Body Weight Regulation in *ob/ob* Mice. *Science* 1995;269(5223):540-543.
21. Martini FH. Fundamentals of Anatomy and Physiology. 7th edition ed. San Francisco: Pearson Education Inc; 2006.
22. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated Regional Sympathetic Nerve Activation by Leptin. *J. Clin. Invest* 1997;100(2):270-278.
23. Begg DP, Woods SC. Handbook of Experimental Pharmacology. In: Joost H-G, editor. Appetite Control; 2012. p. 111-129.

24. Coll AP, Farooqi IS, O'Rahilly S. The Hormonal Control of Food Intake. *Cell* 2007;129(2):251-262.
25. Batterham RL, Bloom SR. The Gut Hormone Peptide YY Regulates Appetite. *Ann N Y Acad Sci* 2003 994:162-168.
26. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metabolism* 2006;4(3):223-233.
27. Broberger C. Brain regulation of food intake and appetite: molecules and networks. *J Intern Med* 2005;258(4):301-327.
28. 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):1714-1719.
29. Kamegai J, Tamura H, Shimizu TI, Shinya, Sugihara H, Wakabayashi I. Central Effect of Ghrelin, an Endogenous Growth Hormone Secretagogue, on Hypothalamic Peptide Gene Expression. *Endocrinology* 2000;141(12):4797-4800.
30. Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. *Gastroenterology* 2007;132(6):2131-2157.
31. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food Intake and the Regulation of Body Weight. *Annu Rev Psychol* 2000;51:255-277.
32. Daniels SR. The Consequences of Childhood Overweight and Obesity. In: *Future Child*; 2006. p. 47-67.
33. Amed S, Dean HJ, Panagiotopoulos C, Sellers EAC, Hadjiyannakis S, Laubscher TA, et al. Type 2 Diabetes, Medication-Induced Diabetes, and Monogenic Diabetes in Canadian Children: A prospective national surveillance study. *Diabetes Care* 2010;33(4):786-791.
34. Canadian Diabetes Association. *Metabolic Syndrome*. In: Toronto: Canadian Diabetes Association,; 2012.
35. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The Relation of Overweight to Cardiovascular Risk Factors Among Children and Adolescents: The Bogalusa Heart Study. *Pediatrics* 1999;103(6):1175-1182.
36. Raj M, Kumar K. Obesity in Children & Adolescents. *Indian J. Med. Res.* 2010;132(5):598-607.
37. American Diabetes Association. Type 2 Diabetes in children and adolescents. *Pediatrics* 2000;105(3):671-680.
38. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting Obesity in Young Adulthood from Childhood and Parental Obesity. *New Eng J Med* 1997;337(13):869-873.
39. Hershberger AM. Responses of Lipolysis and Salivary Cortisol to Food Intake and Physical Activity in Lean and Obese Children. *J Clin Endocr Metab* 2004;89(9):4701-4707.
40. Cavadini C, Siega-Riz MS, Popkin BM. US adolescent food intake trends from 1965 to 1996. *Arch Dis Child* 2000;83(2):18-24.
41. Hanning RM, Woodruff SJ, Lambraki I, Jessup L, Driezen P, Murphy CC. Nutrient intake and Food Consumption among Ontario Students in Grades Six, Seven, and Eight. *C J Public Health* 2007;98(1):12-16.
42. Lau DCW, Douketis JD, Morrison KM, Hramiak IM, Sharma AM, Ur E. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. *Can Med Assoc J* 2007;176(8):S1-S13.
43. Roblin L. Childhood obesity: food, nutrient, and eating-habit trends and influences. *Appl Physiol Nutr Metab* 2007;32(4):635-645.
44. Kretzel L. Ontario school boards lose millions on healthy food. *National Post* 2012 June 26, 2012.
45. Kennedy G. Making Ontario Schools Healthier Places to Learn. In: *Education Mo*, editor.; 2004.
46. Wilkinson Enns C, Mickle SJ, Goldman JD. Trends in Food and Nutrient Intakes by Adolescents in the United States. *Fam Econ Rev* 2003;15(2):15-28.

47. Young LR, Nestle M. The Contribution of Expanding Portion Sizes to the US Obesity Epidemic. *Am J Public Health* 2002;92(2):246-249.
48. Nicklas TA, Yang S-J, Baranowski T, Zakeri I, Berenson G. Eating patterns and obesity in children. *Am J Prev Med* 2003;25(1):9-16.
49. Boumtje P, Huang C, Lee J-Y, Lin B-H. Dietary habits, demographics, and the development of overweight and obesity among children in the United States. *Food Policy* 2005;30(2):115-128.
50. French SA, Story M, Neumark-Sztainer D, Fulkerson JA, Hannan P. Fast food restaurant use among adolescents: associations with nutrient intake, food choices and behavioral and psychosocial variables. *Int J Obesity* 2001;25(12):1823-1833.
51. Gilbert J-A, Miller D, Olson S, St-Pierre S. After-school Snack Intake Among Canadian Children and Adolescents. *Can J Public Health* 2012;103(6):448-452.
52. Jahns L, Siega-Riz AM, Popkin BM. The increasing prevalence of snacking among US children from 1977 to 1996. *J Pediatr* 2001;138(4):493-498.
53. Agriculture and Agri-Food Canada. Consumer Trends: Salty Snack Food in the United States. In: Agriculture and Agri-Food Canada, editor. Ottawa; 2011.
54. Agriculture and Agri-Food Canada. The Canadian Consumer Behaviour, Attitudes and Perceptions Toward Food Products. In: Agriculture and Agri-Food Canada, editor. Ottawa; 2010.
55. Health Canada. Eating Well With Canada's Food Guide. In: Health Canada, editor. Ottawa; 2011.
56. Moffat T, Galloway T. Food Consumption Patterns in Elementary School Children. *Can J Diet Pract Res* 2008;69(3):152-154.
57. Schrage S. Dietary Calcium Intake and Obesity. *J Am Board Fam Pract* 2005;18(3):205-210.
58. Astrup A, Chaput J-P, Gilbert J-A, Lorenzen JK. Dairy beverages and energy balance. *Physiol. Behav.* 2010;100(1):67-75.
59. Steijns JM. Dairy products and health: Focus on their constituents or on the matrix? *Int Dairy J* 2008;18(5):425-435.
60. Kelishadi R, Zemel MB, Hashemipour M, Mohsen 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):601-610.
61. Marques-Vidal P, Gonçalves A, Dias CM. Milk intake is inversely related to obesity in men and in young women: data from the Portuguese Health Interview Survey 1998–1999. *Int J Obes* 2005;30(1):88-93.
62. Berkey CS, Rockett HRH, Willett WC, Colditz GA. Milk, Dairy Fat, Dietary Calcium, and Weight Gain. *Arch Pediatr Adolesc Med* 2005;159(6):543-550.
63. Moore LL, Bradlee ML, Gao D, Singer MR. Low Dairy Intake in Early Childhood Predicts Excess Body Fat Gain. *Obesity* 2006;14(6):1010-1018.
64. Blundell J, de Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods. *Obes Rev* 2010;11(3):251-270.
65. Hall WL, Millward DJ, Long SJ, Morgan LM. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr* 2007;99(2):239-248.
66. Anderson G, Moore S. Dietary Proteins in the Regulation of Food Intake and Body Weight in Humans. *J. Nutr.* 2004;134 (4):974S-979S.
67. Anderson GH, Tecimer SN, Shah D, Zafar TA. Protein Source, Quantity, and Time of Consumption Determine the Effect of Proteins on Short-Term Food Intake in Young Men. *J Nutr* 2004;134(11):3011-3015.
68. Mellinkoff S, Frankland M, Boyle D, Greipel M. Relationship Between Serum Amino Acid Concentration and Fluctuations in Appetite. *J Appl Physiol* 1956;8(5):535-538.

69. Boirie Y, Dangin MG, Pierre, Vasson M-P, Maubois J-L, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc. Natl. Acad. Sci.* 1997;94(26):14930–14935.
70. Bowen J. Energy Intake, Ghrelin, and Cholecystokinin after Different Carbohydrate and Protein Preloads in Overweight Men. *Journal of Clinical Endocrinology & Metabolism* 2006;91(4):1477-1483.
71. Hanrahan K, McCarthy AM, Kleiber C, Lutgendorf S, Tsalikian E. Strategies for salivary cortisol collection and analysis in research with children. *Appl Nurs Res* 2006;19(2):95-101.
72. George SA, Khan S, Briggs H, Abelson JL. CRH-stimulated cortisol release and food intake in healthy, non-obese adults. *Psychoneuroendocrinology* 2010;35(4):607-612.
73. Kryski KR, Smith HJ, Sheikh HI, Singh SM, Hayden EP. Assessing stress reactivity indexed via salivary cortisol in preschool-aged children. *Psychoneuroendocrinology* 2011;36(8):1127-1136.
74. Tataranni P, Larson D, Snitker S, Young J, Flatt J, Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* 1996;271(2 Pt 1):E317-E325.
75. Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology* 2001;26(1):37-49.
76. Stimson RH, Johnstone AM, Homer NZM, Wake DJ, Morton NM, Andrew R, et al. Dietary Macronutrient Content Alters Cortisol Metabolism Independently of Body Weight Changes in Obese Men. *Journal of Clinical Endocrinology & Metabolism* 2007;92(11):4480-4484.
77. Duong M, Cohen JI, Convit A. High cortisol levels are associated with low quality food choice in type 2 diabetes. *Endocrine* 2011;41(1):76-81.
78. Sinha R, Jastreboff AM. Stress as a Common Risk Factor for Obesity and Addiction. *Biological Psychiatry* 2013;73(9):827-835.
79. Rosmond R, Holm G, Bjorntorp P. Food-induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men. *Int J Obes* 2000;24(4):416-422.
80. Tornhage C-J. Reference values for Morning Salivary Cortisol Concentrations in Healthy School-aged Children. *J Pediatr Endocr Met* 2002;15(2):197-204.
81. McCarthy AM, Hanrahan K, Kleiber C, Zimmerman MB, Lutgendorf S, Tsalikian E. Normative salivary cortisol values and responsivity in children. *Appl Nurs Res* 2009;22(1):54-62.
82. Stimson RH, Mohd-Shukri NA, Bolton JL, Andrew R, Reynolds RM, Walker BR. The post-prandial rise in plasma cortisol in men is mediated by macronutrient-specific stimulation of adrenal and extra-adrenal cortisol production. *Journal of Clinical Endocrinology & Metabolism* 2013.
83. Netherton C, Goodyer I, Tamplin A, Herbert J. Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology* 2004;29(2):125-140.
84. Rosmalen JGM, Oldehinkel AJ, Ormel J, de Winter AF, Buitelaar JK, Verhulst FC. Determinants of salivary cortisol levels in 10–12 year old children; a population-based study of individual differences. *Psychoneuroendocrinology* 2005;30(5):483-495.
85. Patel BP, Bellissimo N, Tothomas SG, Hamilton JK, Anderson GH. Television Viewing at Mealtime Reduces Caloric Compensation in Peripubertal, But Not Postpubertal, Girls. *Pediatr Res* 2011;70(5):513-517.
86. Baker JH, Thornton LM, Lichtenstein P, Bulik CM. Pubertal development predicts eating behaviors in adolescence. *International Journal of Eating Disorders* 2012;45(7):819-826.
87. Abbassi V. Growth and Normal Puberty. *Pediatrics* 1998;102(Supplement 3):507-511.
88. Tanner JM. Growth and Maturation during Adolescence. *Nutr Rev* 1981;39(2):43-55.
89. Herman-Giddens ME, Bourdony CJ, Dowshen SA, Reiter EO. Assessment of Sexual Maturity Stages in Girls and Boys : Pediatric Research in Office Settings, Department of Research. In: Herman-Giddens ME, Bourdony CJ, Dowshen SA, editors.: American Academy of Pediatrics; 2010.

90. Rapkin AJ, Tsao JCI, Turk N, Anderson M, Zeltzer LK. Relationships among Self-Rated Tanner Staging, Hormones, and Psychosocial Factors in Healthy Female Adolescents. *J Pediatr Adolesc Gynecol* 2006;19(3):181-187.
91. Gregersen NT, Flint A, Bitz C, Blundell JE, Raben A, Astrup A. Reproducibility and power of ad libitum energy intake assessed by repeated single meals. *Am J Clin Nutr* 2008;87(5):1277-1281.
92. 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):382-389.
93. Patel BP, Bellissimo N, Luhovyy B, Bennett LJ, Hurton E, Painter JE, et al. An After-School Snack of Raisins Lowers Cumulative Food Intake in Young Children. *Journal of Food Science* 2013;78(s1):A5-A10.
94. World Medical A. World medical association declaration of helsinki: Ethical principles for medical research involving human subjects. *JAMA* 2013;310(20):2191-2194.
95. Schwartz EB, Granger DA, Susman EJ, Gunnar MR, B. L. Assessing Salivary Cortisol in Studies of Child Development. *Child Dev* 1998;69(6):1503-1513.
96. Anderson G, Catherine NLA, Woodend DM, Wolever TMS. Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *The American Journal of Clinical Nutrition* 2002;76:1023-1030.
97. Luhovyy BL, Akhavan, T., Anderson, G.H. Whey Proteins in the Regulation of Food Intake and Satiety. *Journal of the American College of Nutrition* 2007;26(6):704S-712S.
98. Bendtsen L, Lorenzen J, Bendtsen N, Rasmussen C, Astrup A. Effect of dairy proteins on appetite, energy expenditure, body weight, and composition: a review of the evidence from controlled clinical trials. *Adv Nutr* 2013;4(4):418-438.
99. Bornhorst GM, Singh RP. Bolus Formation and Disintegration during Digestion of Food Carbohydrates. *Comprehensive Reviews in Food Science and Food Safety* 2012;11(2):101-118.
100. Zijlstra N, Mars M, de Wijk RA, Westerterp-Plantenga MS, de Graaf C. The effect of viscosity on ad libitum food intake. *Int J Obes* 2007;32(4):676-683.
101. Tieken S, Leidy H, Stull A, Mattes R, Schuster R, Campbell W. Effects of Solid versus Liquid Meal-replacement Products of Similar Energy Content on Hunger, Satiety, and Appetite-regulating Hormones in Older Adults. *Horm Metab Res* 2007;39(5):389-394.
102. Stull AJ, Apolzan JW, Thalacker-Mercer AE, Iglay HB, Campbell WW. Liquid and Solid Meal Replacement Products Differentially Affect Postprandial Appetite and Food Intake in Older Adults. *J Am Diet Assoc* 2008;108(7):1226-1230.
103. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. *Int J Obes* 2000;24(6):794-800.
104. Carbonnel F, Lemann M, Rambaud JC, Mundler O, Jian R. Effect of the energy density of a solid-liquid meal on gastric emptying and satiety. *Am J Clin Nutr* 1994;60(3):307-311.
105. Almiron-Roig E, Flores SY, Drewnowski A. No difference in satiety or in subsequent energy intakes between a beverage and a solid food. *Physiol. Behav.* 2004;82(4):671-677.
106. Mourao DM, Bressan J, Campbell WW, Mattes RD. Effects of food form on appetite and energy intake in lean and obese young adults. *Int J Obes* 2007;31(11):1688-1695.
107. Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, et al. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *Am J Physiol Gastrointest Liver Physiol* 2001;280(6):G1227-1233.
108. Oesch S, Rüegg C, Fischer B, Degen L, Beglinger C. Effect of gastric distension prior to eating on food intake and feelings of satiety in humans. *Physiology & Behavior* 2006;87(5):903-910.
109. Wang G-J, Tomasi D, Backus W, Wang R, Telang F, Geliebter A, et al. Gastric distention activates satiety circuitry in the human brain. *NeuroImage* 2008;39(4):1824-1831.

110. Kissileff HR, Carretta JC, Geliebter A, Pi-Sunyer FX. Cholecystokinin and stomach distension combine to reduce food intake in humans. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R992–R998.
111. Zijlstra N, de Wijk R, Mars M, Stafleu A, de Graaf C. Effect of bite size and oral processing time of a semisolid food on satiety. *Am J Clin Nutr* 2009;90(2):269-275.
112. Chapelot D, Payen F. Comparison of the effects of a liquid yogurt and chocolate bars on satiety: a multidimensional approach. *Br J Nutr* 2009;103(5):760.
113. Hogenkamp PS, Mars M, Stafleu A, de Graaf C. Repeated consumption of a large volume of liquid and semi-solid foods increases ad libitum intake, but does not change expected satiety. *Appetite* 2012;59(2):419-424.
114. Kissileff H. Effects of physical state (liquid-solid) of foods on food intake: procedural and substantive contributions. *The American Journal of Clinical Nutrition* 1985;42:956-965.
115. 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 Obesity* 2000;24(1):38-48.
116. Blundell J, de Graaf K, Finlayson G, Halford JCG, Hetherington M, King N, et al. Measuring Food Intake, Hunger, and Satiety in the Laboratory. In: Allison DB, Baskin ML, editors. *Handbook of assessment methods for eating behaviors and weight-related problems : measures, theory, and research*. Thousand Oaks, Calif. [u.a.]: Sage; 2009.
117. Health Canada. Draft Guidance Document - Satiety Health Claims on Food. In: Sciences BoN, editor.: Health Canada; 2012.
118. Hogenkamp PS, Mars M, Stafleu A, de Graaf C. Intake during repeated exposure to low- and high-energy-dense yogurts by different means of consumption. *Am J Clin Nutr* 2010;91(4):841-847.
119. Tsuchiya A, Almiron-Roig E, Lluch A, Guyonnet D, Drewnowski A. Higher Satiety Ratings Following Yogurt Consumption Relative to Fruit Drink or Dairy Fruit Drink. *J Am Diet Assoc* 2006;106(4):550-557.
120. 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. *Nutrition Journal* 2013;12(1):71.
121. Hollis JH, Mattes RD. Effect of Increased Dairy Consumption on Appetitive Ratings and Food Intake. *Obesity* 2007;15(6):1520-1526.
122. Dougkas A, Minihiene AM, Givens DI, Reynolds CK, Yaqoob P. Differential effects of dairy snacks on appetite, but not overall energy intake. *Br J Nutr* 2012;108(12):2274-2285.
123. Harper A, James A, Flint A, Astrup A. Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. *Br J Nutr*. 2007;97(3):570-583.
124. Maersk M, Belza A, Holst JJ, Fenger-Grøn M, Pedersen SB, Astrup A, et al. Satiety scores and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk and with non-caloric soft drink: a controlled trial. *Eur J Clin Nutr* 2012;66(4):523-529.
125. Dove ER, Hodgson JM, Puddey IB, Beilin LJ, Lee YP, Mori TA. Skim milk compared with a fruit drink acutely reduces appetite and energy intake in overweight men and women. *Am J Clin Nutr* 2009;90(1):70-75.
126. Soenen S, Westerterp-Plantenga MS. No differences in satiety or energy intake after high-fructose corn syrup, sucrose, or milk preloads. *Am J Clin Nutr* 2007;86(6):1586-1594.
127. Panahi S, Luhovyy BL, Liu TT, Akhavan T, El Khoury D, Goff HD, 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):154-161.
128. Akhavan T, Luhovyy BL, Anderson GH. Effect of drinking compared with eating sugars or whey protein on short-term appetite and food intake. *International Journal of Obesity* 2010;35(4):562-569.

129. El Khoury D, Brown P, Smith G, Berengut S, Panahi S, Kubant R, et al. Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin. *Clin Nutr* 2013;S0261-5614(13).
130. DellaValle DM, Roe LS, Rolls BJ. Does the consumption of caloric and non-caloric beverages with a meal affect energy intake? *Appetite* 2005;44(2):187-193.
131. Douglas SM, Ortinau LC, Hoertel HA, Leidy HJ. Low, moderate, or high protein yogurt snacks on appetite control and subsequent eating in healthy women. *Appetite* 2013;60(1):117-122.
132. Ranawana DV, Henry CJK. Are caloric beverages compensated for in the short-term by young adults? An investigation with particular focus on gender differences. *Appetite* 2010;55(1):137-146.
133. Davy BM, Van Walleghen EL, Orr JS. Sex differences in acute energy intake regulation. *Appetite* 2007;49(1):141-147.
134. Shide DJ, Rolls BJ. Information About the Fat Content of Preloads Influences Energy Intake in Healthy Women. *J Am Diet Assoc* 1995;95(9):993-998.

Appendices

Appendix 1: Nutrition Information for Dietary Treatments

Energy & Macronutrients per treatment	2% Milk	Cheese	Cookies	Potato Chips	Yogurt
Weight (g)	346.2	63	38.6	32.1	200
Energy (kcal)	180	180	180	180	180
Total Fat (g)	6.9	12.0	7.7	11.6	0
Protein (g)	12.5	18.0	1.3	1.9	17.1
Carbohydrate (g)	16.6	0.0	27.0	16.7	26.3
Sugars (g)	16.6	0	15.4	0	24
Fibre (g)	0.0	0.0	1.3	0.6	2
Sodium (g)	166.2	420.0	218.6	212.1	62.9

Appendix 2: Ethics Clearance from the University Research Ethics Board at MSVU



University Research Ethics Board

UNIVERSITY RESEARCH ETHICS BOARD

Certificate of Research Ethics Clearance

File #: 2013-014
Title of project: *The Effect of Dairy and Non-dairy Products on Satiety and Food Intake in Children*
Researcher(s): Athena Chi-Yan
Supervisor (if applicable): Bohdan Luhovyy
Co-Investigators: n/a
Version : 1

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

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

Dr. Daniel Séguin, Chair University Research Ethics Board	<u>July 15, 2013</u> Effective Date <i>[Expires: July 14, 2014]</i>
--	---

Appendix 3a Newspaper Advertisements

	Mount Saint Vincent University Department of Applied Human Nutrition	
ATTENTION: PARENTS OF CHILDREN AGED 9-14 YEARS		
We are conducting a research study to learn more about milk products in child nutrition.		
REQUIREMENTS: 9-14 year old boys & girls, Healthy, have been born at term and not be taking medication		
INVOLVES: screening with the information session + 5 weekend sessions Children will be asked to drink or eat common snacks. Lunch will be provided.		
As a reward for taking part: The child will receive a \$10 gift certificate for each session Plus \$5 per visit for parents for travel reimbursement		
Please contact us at: (902) 266-8950 or Email us at: appetite.study@msvu.ca		

Appendix 3b Online Advertisements

	Mount Saint Vincent University Department of Applied Human Nutrition	
ATTENTION: PARENTS OF CHILDREN AGED 9-14 YEARS		
We are conducting a research study to learn more about milk products in child nutrition.		
REQUIREMENTS: 9-14 year old boys & girls, Healthy, have been born at term and not be taking medication		
INVOLVES: screening with the information session + 5 weekend sessions Children will be asked to drink or eat common snacks. Lunch will be provided.		
As a reward for taking part: The child will receive a \$10 gift certificate for each session Plus \$5 per visit for parents for travel reimbursement		
Please contact us at: (902) 266-8950 or Email us at: appetite.study@msvu.ca		

MSVU Department of **Applied Human Nutrition** is conducting a research study to learn about milk products in children 9-14 years old. Children will be asked to drink or eat common snacks. Lunch will be provided. If interested, please contact us at: **902-266-8950** or email us at: appetite.study@msvu.ca

Appendix 3c Posted Advertisements



**ATTENTION PARENTS OF
9 TO 14 YEARS OLDS!**

*We are currently conducting several nutrition studies
to better our understanding of how to develop
healthy eating habits in children*

*Studies take place on weekend mornings
at the Mount or IWK. It's a
great way to meet other kids!*

*Your child will receive a
personalized copy of
Canada's Food Guide*

*Breakfast and lunch
provided!*



*As a reward for taking part, at each session your child will receive a gift card
of her/his choice. Parents will also be reimbursed for their travel.*

CONTACT US FOR MORE INFORMATION



902 457 6378



Appetite-Study@msvu.ca



Mount Saint Vincent University
Department of Applied Human Nutrition



ATTENTION: PARENTS OF CHILDREN AGED 9-14 YEARS

We are conducting a research study to learn more about milk products in child nutrition.

REQUIREMENTS: 9-14 year old boys & girls,
Healthy, have been born at term and not be taking medication

INVOLVES: screening with the information session
+ 5 weekend sessions
Children will be asked to drink or eat common snacks.
Lunch will be provided.

As a reward for taking part:
**The child will receive a \$10 gift certificate for
each session**
Plus \$5 per visit for parents for travel reimbursement

Please contact us at: (902) 266-8950

or

Email us at: appetite.study@msvu.ca

Appendix 4a Telephone Screening Form

Mount St Vincent University: Department of Applied Human Nutrition

Pre-meal Snacks, Satiety and Food Intake in Children:

Name: _____

Age: _____ years

Date of Birth: (d/m/y) _____ **Term baby?** Yes / No **Normal Birth Weight?:** Yes / No

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?: _____

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?: _____

Does your child have any behavioral or emotional problems? Yes / No

(If yes explain: _____)

Include in study? Yes / No

Appointment scheduled for: (date and time) _____

Investigator/Date screened: _____

Appendix 4b Participant Exclusion List

1. Age outside of the 9-14.5 years old age range.
2. Inability to consume treatment and test meal food items, which include milk, strawberry yogurt, stringed cheese, chips, Oreo Mr.Christie's cookies (Kraft Canada) and chips, whether it is due to personal preference or medical reasons, for example lactose intolerance.
3. BMI \leq the 5th percentile, as anything under that is considered underweight.
4. Recent weight fluctuations (loss/gain).
5. Those who do not usually consume breakfast.
6. Other than vegetarianism, those who follow special diets will not be considered for this study.
7. Those with medical/health problems and those who are currently taking medication.
8. Those with learning difficulties or with emotional/behavioural problems.
9. Those who do not speak/understand English, as the consent forms are only written in English.
10. Those who were not born at term.

Appendix 5a Recruitment Letter



Excellence • Innovation • Discovery

**Department of Applied Human Nutrition
312 Evaristus Hall, 166 Bedford Highway
Halifax, NS B3M 2J6
CANADA**

**The Effect of Solid, Semi-Solid and Fluid Dairy Products on Blood Glucose, Food Intake and Satiety of Normal Weight and Overweight/Obese Children
(Sessions WITHOUT clinical testing)**

Recruitment Letter for Parents

Dear Parent

A team of researchers from Mount Saint Vincent University and the University of Toronto are investigating the effects of milk products on energy intake regulation in children and young adolescents. The ultimate goal of this research is to find ways to address the problems of overeating and obesity that are becoming a concern among those people involved in the long term health of Canadians.

We are asking the parents of girls and boys 9 – 14 years old to allow their daughter/son to take part in a research study. On five separate mornings your child will consume a drink or snack followed by a pizza lunch 120 minutes later. In addition, we will collect saliva samples and your child will be asked to complete questionnaires. To determine your son/daughter's eligibility you will be asked first to attend a screening/information session. The study will take place on five weekend, holiday or summer mornings at 365 Evaristus Hall, Department of Applied Human Nutrition (166 Bedford Highway).

There are criteria for participation that you need to be aware of, the child must:

- be between 9 to 14 years of age, and
- be healthy, and have been born at term, and
- not be taking medications.

As a reward for taking part, at each session the child will be given a gift certificate for \$10. In addition parents will be reimbursed for travel/parking expenses (\$5).

This study has been fully approved by the Research Ethics Board.

If you would like your son/daughter to participate, or to get further information beyond that provided in this letter, please contact Evelyn Hurton, [REDACTED] at Mount Saint Vincent University (Department of Applied Human Nutrition).

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

Thank you for your support in this important research.

Sincerely,
Drs. Bohdan Luhovyy and Nick Bellissimo

Appendix 5b Parental Consent Form



Excellence • Innovation • Discovery

**Department of Applied Human Nutrition
312 Evaristus Hall, 166 Bedford Highway
Halifax, NS B3M 2J6
CANADA**

**The Effect of Solid, Semi-Solid and Fluid Dairy Products on Blood Glucose, Food Intake and Satiety of Normal Weight and Overweight/Obese Children
(Sessions WITHOUT clinical testing)**

Study Information Sheet and Parent's Consent Form

Investigators:

Dr. Bohdan Luhovyy, Investigator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: [REDACTED]
Email: [REDACTED]

Dr. Nick Bellissimo, Investigator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: [REDACTED]
Email: [REDACTED]

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

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

Ms. Tove Armstrong, Research Coordinator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: [REDACTED]
Email: [REDACTED]

Ms. Evelyn Hurton, Research Coordinator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: [REDACTED]

Email: [REDACTED]

Ms. Kim Allen, Research Coordinator
Department of Applied Human Nutrition, Mount Saint Vincent University

Phone: [REDACTED]
Email: [REDACTED]

Purpose of Research:

The purpose of this study is to determine the effects of dairy products on food intake and blood sugar regulation in 9-14 year-old children. This experiment is being conducted through the Departments of Applied Human Nutrition at Mount Saint Vincent University and Nutritional Sciences at the University of Toronto by Dr. Bohdan Luhovyy, Dr. Nick Bellissimo and Dr. G. Harvey Anderson. Your son/daughter will be required to attend five experimental sessions conducted over a 5-week period and one screening session to measure physiological parameters for a total of 6 visits (1 screening session + 5 experimental sessions) to the Mount Saint Vincent University campus. Experimental sessions will last a maximum of 3 hours.

The purpose of our research is to develop an understanding of factors affecting the control of food intake and blood glucose in children. Knowing the determinants of the regulation of food intake and blood glucose in children will allow us to develop strategies and recommendations for the prevention of obesity and diabetes.

Procedure:

Screening: For those parents who express interest in having their son/daughter participate, some information about the child will be requested by telephone. If the child was born at term, is healthy and does not receive any medications, a screening session will be arranged.

During the screening session, the researcher will explain the full details of the study. Parents that give consent to have their son/daughter participate will sign a consent form. The parent will receive copies of the consent form and of the study information sheet. If the child wishes to participate and signs a children's assent form, his/her weight, height, and body fat using painless techniques, will be measured.

The boys and girls will then be asked to rank their preference for pizza that will be served as the lunch meal at each session.

The Children's physical activity and eating habits will be assessed with Physical Activity Questionnaire and the Dutch Eating Behaviour Questionnaire.

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. The children will be asked to circle the number on the side of the picture that best represents them. 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. In order to assess pubertal stage, the children will be asked to complete a questionnaire about puberty and changes in their bodies. Depending on the sex of the child, the children will be presented with cartoon pictograms of different stages of physical/sexual development (e.g., breast size, pubic hair, genitalia) and the children will be asked to pick the picture that best represents their stage of puberty. These pictograms have been used extensively in children. If for any reason the children are not willing to participate, they have the option of asking their parents to answer the questionnaire and select the pictograms for them. The children may decline the pubertal staging if they wish. Parents are welcome to discuss the reasons for including Tanner stages as part of the study or any comment or concerns with Dr. Jill Hamilton at [REDACTED]

Body Composition Assessment: The method of skinfold thickness technique will be used to estimate the amount of muscle and fat tissue in your child's body.

Skinfolds: The skinfold thickness technique is performed by pinching the skin between the thumb and forefinger and placing calipers on the fold measuring the width of thickness of the two layers of skin and subcutaneous fat underneath. The assumptions underlying the rationale of measuring skinfold thickness are that skinfold thickness is an adequate measure of subcutaneous fat (fat under the skin) and that there is a defined relationship between subcutaneous fat and total body fat.

Appetite, Food Intake and Salivary Cortisol Assessment: The boys/girls who participate in this study, will be requested to go to the Evaristus Building, Department of Applied Human Nutrition, Mount Saint Vincent University, for five individual morning sessions. These sessions will be held on weekends, over five weeks. The children will be brought to the laboratory and returned home by parents only.

On each of the five test days, the children will have a standardized breakfast of cereal, milk and juice at home, either at 7:00 am or 7:30 am (the time will be consistent for each child). The children will arrive at the Evaristus Building, either at 9:30 am or 10:00 am (but consistent throughout for each child).

Children will fast for 12 hours before breakfast and after breakfast until their arrival, except for water, which will be allowed up to one hour before their arrival.

Upon arrival, children will be given one of the following products at each session: milk, yogurt, mozzarella cheese, cookies, or potato chips). Children will be asked to eat or drink the whole portion of the product that will be provided. Then in two hours, we will serve McCain pizza and children will be told that they may eat as little or as much pizza as they like. The amount of food eaten by each child will be measured.

The boys/girls will also be asked to complete scales on which they will place a pencil mark to describe their desire to eat (“Very weak” to “Very strong”), hunger (“Not hungry at all” to “As hungry as I’ve ever felt”), fullness (“Not full at all” to “Very full”), how much food they could eat (“A large amount” to “Nothing at all”). They will also be asked to complete similar scales on how much they like the drinks and the pizza. They will complete these scales during the information session, in order to become familiar with the test instruments.

Samples of saliva will be collected for analysis of cortisol during the sessions where you child consumes yogurt or cookies as an indicator of the child’s stress level. Under our supervision and assistance, your child will be asked to places the synthetic swab in the mouth and chew it for about 45 seconds.

The children will be fully supervised during the study sessions. They will be involved in age appropriate entertainment (as distraction) e.g.: reading, puzzles, cards, before lunch. The study session will end following the pizza meal.

Confidentiality:

Records relating to participants 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 will be entered into Microsoft Excel files, available only to investigators. Each participant will have a file, also only available for investigators. 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.

Risks:

There is very little risk related to this study. The provided snacks and pizza are commercially available and safe for human consumption. In addition, the pizza that children will be asked to consume are prepared hygienically in the kitchen at the time of the session and present minimal risk. 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.

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.

Questions and further information:

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

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Dr. Bohdan Luhovyy ([REDACTED]) or Dr. Nick Bellissimo ([REDACTED])

Dissemination of findings:

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

Consent:

I acknowledge that the research procedures described above and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. I know that I may ask additional questions now or in the future. I am aware that participation in the study will not involve any health risk to my child.

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 prejudice.

Upon completion of each study session, my child will receive a gift certificate for \$10. The final summary and results of the study will be available for me to pick up from the Department of Applied Human Nutrition, Mount Saint Vincent University. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter's identity.

I hereby consent for my child, _____, to participate in this study.

(Name of parent or guardian)

(Signature of parent or guardian)

(Name of witness)

(Signature of witness)

Date: _____ (dd/mm/yy)

Appendix 5c Participant Consent Form



Excellence • Innovation • Discovery

**Department of Applied Human Nutrition
312 Evaristus Hall, 166 Bedford Highway
Halifax, NS B3M 2J6
CANADA**

**The Effect of Solid, Semi-Solid and Fluid Dairy Products on Blood Glucose, Food Intake and Satiety of Normal Weight and Overweight/Obese Children
(Sessions WITHOUT clinical testing)**

Children's Assent Form

This study will help to find out how good various snacks and drinks are for children's health. My weight, height, and body fat will be measured without pain during the screening visit. I will be asked to fill out a questionnaire that is related to my stage of puberty (changes in my body as I grow up). I will also be asked to look at some drawings and pick the one that looks most like me. If I am a girl, I will be asked to answer questions about whether I have started my menstrual cycle. I can ask my parents to answer these questionnaires and pick the drawing for me. I will also be asked to drink milk or eat a snack, complete special scales to show if I am hungry or full and have saliva samples taken. I will also be provided with a pizza lunch during each study session in the Department of Applied Human Nutrition, Mount Saint Vincent University. All the experimental sessions will be on weekends, school holidays or summer break, so I don't need to be absent from school.

I know that my participation in the study will not involve any health risk to me.

I will be asked to come for the study five times for approximately three hours but if at any time I decide to stop participating, that will be O.K and I have the choice to not answer any question at any time. I understand that the information related to me will be securely stored and not be given to anyone from outside who is not involved with this study. I know that I will receive a gift certificate for \$10 after completion of each study session, as a "thank you" for my participation.

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

_____ Signature

Name of the person who obtained assent:

Appendix 6a Puberty Questionnaire

Puberty Questionnaire (Self-administered)

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?

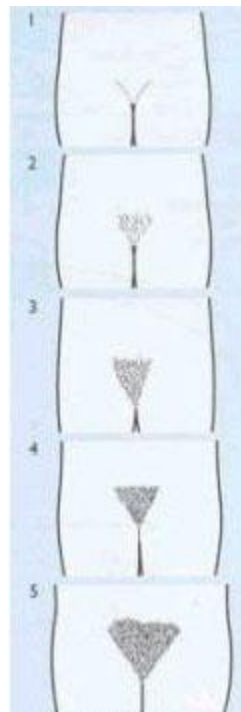
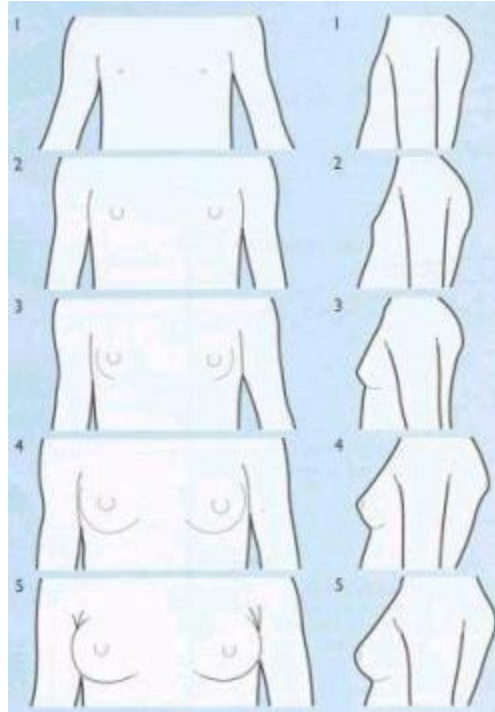
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway

***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.**

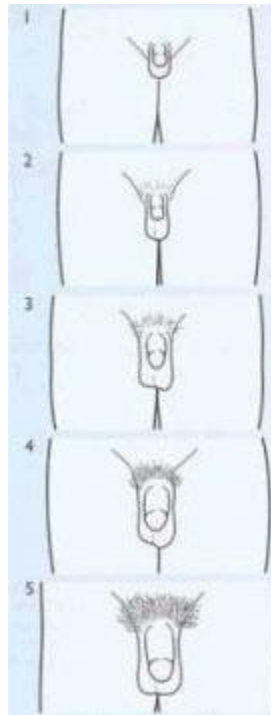
Tanner stage exam be MD for boys and premenarchial girls. If p refuses exam, self-stage with cartoons and tanner beads (for boys).

Appendix 6b Tanner Staging Questionnaires

Tanner Staging (Females)



Tanner Staging (Males)



Appendix 6c Menstrual Cycle Questionnaire

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

If you answered no, you are finished this questionnaire.

If you answered yes, please complete the following questions.

3. How old were you when you had your first period?
I was _____ years old when I had my first period.
4. Do you remember the day/month of your first period? Yes/No
5. If you answered “yes”, what was the date of your first period? _____
6. How long is your average menstrual cycle? (from the beginning of menstrual flow [menses] to the beginning of the next menstrual flow [menses])
My average cycle length is _____ days.
7. Currently, for how many days do you typically experience menstrual flow each cycle?
____ 1 day ____ 2 days ____ 3 days ____ 4 days ____ 5 days ____ > 5+ days
8. In the past 3 months, estimate how many menstrual cycles you have had?
I have had _____ cycles in the past 3 months
9. In the past 6 months, estimate how many menstrual cycles you have had?
I have had _____ cycles in the past 6 months
10. In the past 9 months, estimate how many menstrual cycles you have had?
I have had _____ cycles in the past 9 months
11. In the past 12 months, estimate how many menstrual cycles you have had?
I have had _____ cycles in the past 12 months
12. How would you characterize your menstrual flow in the first two days of menses?

Circle one: Heavy Moderate Light

13. Do you experience cramps during menses?

Circle One: Always Sometimes Never

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

Circle one: Always Sometimes Never

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

Circle one: Always Sometimes Never

Appendix 6e Dutch Eating Behaviour Questionnaire

Subject and test details

Name: _____

Date of birth: _____

Age: _____

Gender: male female

Today's date: _____

2. Your weight, height, etc.

5. 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		

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 7a VAS Cover Sheet

Feeding Session Cover Sheet

Department of Applied Human Nutrition, Mount Saint Vincent University

Food Intake Control in Children

Subject ID:

Session:

Date:

Baseline Questionnaire (to be asked by investigator)

1. Have you had the standardized breakfast this morning?

YES/NO

2. At what time did you have the standardized breakfast?

3. Have you had anything to eat or drink for 10 – 12 hours before breakfast?

YES/NO

If yes, please describe
briefly

4. Have you had anything to eat or drink after breakfast before arriving here?

YES/NO

If yes, please describe

briefly

5. Are you taking any medication?

YES/NO

If yes, please describe
briefly

Comments/Notes:

What time did you go to
bed? _____

What time did you wake
up? _____

Appendix 7b VAS Motivation to Eat

Time =

Visual Analogue Scale Motivation to Eat

DATE: _____

NAME: _____

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 _____ As hungry as I have ever felt
at all

3. How full do you feel?

NOT Full _____ VERY Full
at all

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

NOTHING _____ A LARGE amount
at all

Appendix 7c VAS Physical Comfort

Time =

Visual Analogue Scale Physical Comfort

DATE: _____

NAME: _____

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 well do you feel?

NOT
well
at all

VERY
Well

Appendix 7d VAS Sweetness

Time =

Visual Analogue Scale Sweetness

DATE: _____

NAME: _____

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 sweet have you found the drink/food?

NOT _____ Very
at all sweet
sweet

Appendix 7e VAS Pleasantness

Time =

Visual Analogue Scale Pleasantness

DATE: _____

NAME: _____

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 _____ Very
at all pleasant
pleasant

Appendix 8 Detailed description of the method of saliva collection for salivary cortisol assay

It should be noted that during all each sessions, one student/volunteer will be in charge of collecting saliva samples and supervising the participants, only when collecting saliva samples.. This will help eliminate human error that could occur if more than one person is involved with collecting saliva, or if one person has the task of supervising and collecting saliva. The student/volunteer in charge of saliva collection will wear latex gloves at all times and is responsible for making sure that the centrifuge is set to the appropriate settings. The saliva collection will only happen in an area of the lab that is sectioned off and is designated for saliva collection only. Only students/volunteers who have undergone pipetting and centrifuge training with Dr Luhovyy will be considered for this task.

Protocol for the collection of saliva is as follows:

1. Participants will be asked to sanitize their hands before and after each sample collection.
2. All Salivette[®] tubes will be labelled and dated with the participant ID, date, treatment, time point and session number.
3. Participant will be asked to remove the blue top of their Salivette[®] tube, remove the cotton swab and place the swab in their mouth and chew. The student/volunteer in charge of collecting saliva for that session will then count to 45 seconds and afterward, the participants will be asked to remove the swab from their mouth and place it back into the Salivette[®] tube and replace the blue cap to seal the Salivette[®] tube.
4. The Salivette[®] tubes are to be centrifuged for 2 minutes at 1,000 x g and at 4°C.
5. The tubes will separate particles and mucus, which will collect in the specially designed tip of the Salivette[®] tube, from the saliva.
6. The saliva will be pipetted into pre-labelled microtubes (Specifications: volume capacity of 0.5mL; temperature range -90 to 140°C storage; 0 to 40°C centrifuging; maximum RCF: 20,000 g; VWR Cat # 89000-010) that will be placed on an ice bath until all saliva samples have been collected. All samples must be frozen at -20°C or lower within 4 hrs of baseline collection. Repeated freeze-thaw is not desired and therefore will be avoided. The cap of the microtubes will have a number that corresponds to the label on the Salivette[®] tube. The body of the microtubes that will have the same labelling system on the body of the Salivette[®] tube. Microtubes will be placed in a moisture-resistant laminated cardboard box with a lid and an insert (dimension: 135 x 135 x 34 mm for 0.5 ml tubes; capacity format: 100 / 10 x 10; Sarstedt Cat # 95.64.923). Samples “A” and “B” will be placed in separate boxes.
7. Each saliva sample will be placed into 2 microtubes, therefore there will be an “A” and a “B” sample, both of which will contain the same sample from the same Salivette[®] tube. This is just to provide extra caution should something happen to a sample.
8. A log will be used to ensure accuracy, as well as to provide a place where mistakes can be recorded, if needed.
9. The Salivette[®] tube will be decontaminated with a bleach solution. Pipette tips are to be decontaminated with at least 70% ethanol alcohol.

Sample Log

Sample #	Participant's ID	Date of Sample Collection	Treatment	Session #	Time Point	Box #	Notes
168A	OV-08	08/08/14	F	4	2	1	

Salivette® Tube Labelling

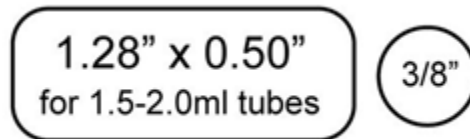


Salivette® Tubes will be hand-labelled with participant ID, date, treatment, time point and session number using a permanent marker on the provided label

Microtube Labelling

Labels for the microtubes will be printed by a laser printer in the lab and placed on the microtubes at least the day before each session.

Rectangular labels will go around each microtube with the following information: Participant's ID, date, treatment, session and time point. These labels are resistant up to -196°C and can be ordered from Electron Microscopy Sciences (Cryo-Clear™ Laser Labels, Cat # 77576-C)



Round top labels from Laser Cryo-Tags Tough-Spot Set (Cat # 77574-W) will have a number and the letter "A" or "B". This number will help in identifying the samples in their storage boxes.

For example: "168A" or "168B"

For example: OV-08, 08/08/14, F, 4, 2

Appendix 9 Nutrition Information for Pizza Meal

	Pepperoni	3 Cheese
Weight(g)	87	81
Calories	180	180
Fat(g)	6	6
Saturated(g)	2.5	2.5
Trans(g)	0.1	0.1
Cholesterol(mg)	15	15
Sodium(mg)	400	360
Carbohydrate(g)	23	22
Fiber(g)	2	2
Sugar(g)	4	4
Protein(g)	9	9

Appendix 10 Salivary Cortisol ELISA Procedure

1. Prior to starting:
 - a. Defrost samples
 - b. Set orbital shaker to 500-900 rpm
 - c. Turn on centrifuge and spin defrosted samples at 1500 x g for 10 minutes, then bring samples to room temperature
 - d. Prepare Cortisol-HRP working reagent:
 - i. Mix 0.5mL of Cortisol-HRP concentrate and 4.5mL of Cortisol-HRP conjugate buffer
 1. Mixture is light sensitive and should be discarded if not used within 36 hours
 - e. Prepare wash solution:
 - i. Mix 25mL of 10x Wash Solution ELISA #1 with 225mL of DI water
 - f. Set microplate washer to wash 3 times using 300µl of wash fluid
 - g. Set microplate reader to 450nm
2. Pipette 50µl of the following into Plate:
 - a. ELISA Calibrators (0, 0.1, 0.3, 1.0, 3.0, 10.0 and 30.0 ng/mL)
 - i. 7 wells, run in duplicates
 - b. Controls (0.993ng/mL and 21.5ng/mL)
 - i. 2 wells, run in singlet
 - c. Saliva samples
 - i. Run in duplicates
 - ii. Follow diagram with respect to which samples are to be used
3. To all wells, add 50µl of Cortisol-HRP working reagent (solution that was mixed prior to starting ELISA) to all wells
4. To all wells, add 50µl of Cortisol ELISA antibody
5. Cover plate with plastic sealer and incubate for 1 hour at room temperature
 - a. Place on a microplate orbital shaker set at 500-900 rpm
6. Decant contents of the wells
7. Wash plate 3 times with 300µl of diluted wash solution
8. Invert microplate and tap dry
9. Pipet 100µl of Colour development reagent ELISA #1 into each well
10. Briefly shake manually. Incubate for 30minutes at room temperature.
11. Pipet 100µl of Stopping Solution ELISA #1 into each well
12. Briefly shake manually. Colour will change from blue to yellow.
13. Read at 450nm within 30 minutes