Mount Saint Vincent University  
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Effect of Sugars-Sweetened Commercial Beverages on Subjective Appetite, and Short-Term Food Intake Regulation in Normal Weight and Overweight/Obese 9 to 14 Year Old Girls

by

Lorianne Bennett

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Abstract

The following study was conducted to examine the hypothesis that 1% chocolate milk suppresses short-term food intake (FI) to a greater extent than other sugars containing beverages, but the effect will be diminished in overweight and obese (OW/OB) girls. The objective was to determine the effect of isovolumetric drinks of (350ml) 1% chocolate milk, fruit drink, and cola 60 min before a pizza meal on subjective appetite, and short-term FI compared to a water control in 9-14 year old normal weight (NW) and OW/OB girls. A decrease in FI after 1% chocolate milk occurred in NW (n=12) but not OW/OB (n=11) girls. None of the other beverages suppressed FI in either group. In the pooled sample (n=23) a decrease in FI occurred after cola and 1% chocolate milk but not fruit drink, which suggests macronutrient composition was the major determinant on FI suppression. Lower prospective food consumption (PFC) and desire to eat (DTE) scores in NW girls after 1% chocolate milk, when corrected for the energy content of the preload, suggests that 1% chocolate milk is a potent regulator of appetite and FI in NW girls. Subjective appetite, body composition, or cognitive restraint, were not strong determinants of FI. In conclusion, most sugars containing beverages suppressed FI, but the response was affected by macronutrient composition, energy content of the beverages, and body weight status.
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<tr>
<td>AA</td>
<td>Average Appetite</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>BG</td>
<td>Blood glucose</td>
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<td>BIA</td>
<td>Bioelectric Impedance Analysis</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<td>CC</td>
<td>Caloric Compensation</td>
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<td>CCK</td>
<td>Cholecytokinin</td>
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<tr>
<td>DEBQ</td>
<td>Dutch Eating Behaviour Questionnaire</td>
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<tr>
<td>DXA</td>
<td>Dual energy x-ray absorptiometry</td>
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<tr>
<td>FFM</td>
<td>Fat-free mass</td>
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<tr>
<td>FI</td>
<td>Food Intake</td>
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<td>FM</td>
<td>Fat Mass</td>
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<tr>
<td>GI</td>
<td>Glycemic Index</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-Like Peptide-1</td>
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<tr>
<td>HFCS</td>
<td>High-fructose corn syrup</td>
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<td>LDL</td>
<td>Low-Density Lipoprotein</td>
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NW Normal weight
O Obese
OW Overweight
P Physical Activity
PYY Peptide Tyrosine Tyrosine
PFC Prospective Food Consumption
S Standard error of mean
SSB Sugar sweetened beverages
T Television Viewing
V Visual analogue scale
VAS
Chapter 1. Introduction
1.0. Introduction

The prevalence of obesity has exploded worldwide in children as well as in adults in the past two decades. In 2001, the associated cost of obesity was estimated at $4.3 billion in Canada, with 50% of adults and 30% of children classified as overweight or obese [1]. Obese children are at high risk of becoming obese adults and suffering from chronic diseases including cardiovascular disease, high blood pressure, stroke and type II diabetes which persist into adulthood, adding to the health care burden of the unhealthy population. Although environmental factors have been the overwhelming focus of research on the causative factors of obesity [2] less is known about the physiological mechanisms of intake control in children. It is unclear if obesity develops in susceptible individuals because physiological mechanisms of food intake control are compromised first or if these are simply overridden by the environment and become compromised [3]. Although, only a handful of studies have investigated intake regulation in boys by measuring food intake (FI) at test meals after caloric beverages, the research literature is even more limited in girls.

Increased availability and consumption of commercially sweetened beverages has paralleled the increase in childhood obesity as the increase has occurred, and this association has been used as evidence in support of a causal relationship. However young children 3-5 years show excellent compensation for calories in sugars in contrast to the hypothesis that sugars in solution by-pass regulatory systems of FI [4]. In addition to the increase in consumption of sugars containing beverages, a decrease in the consumption of cow’s milk has been reported [5] and milk consumption is associated with healthier body weight in kids. The replacement of milk with sugary drinks may increase total energy intake as the protein content and other bioactive components in milk, may lead to a greater effect on satiety than drinks containing carbohydrate
alone [6]. Previous research in children has shown that NW boys reduced FI to a greater extent after whey protein than glucose beverages, but the effect was time dependent [7]. Further research is required to determine the role of macronutrients on FI when consumed in their commercially available forms. This will allow for practical application of research findings and recommendations for reducing short-term FI. This study will provide insight into how macronutrient composition of the preload treatments and body composition affect FI in 9-14 y old girls.
Chapter 2. Literature Review
2.1. Introduction

The literature review provides the relevant background for the proposed study and is comprised of six sections. First a description of childhood obesity including diagnosis, prevalence, causes, and consequences is provided. The second section describes the effect of different macronutrients, on FI regulation. The physiological control of FI is then examined including a review of literature on short-term and long-term gastrointestinal hormones. In the sections that follow the effect of obesity and sex on FI are described. The final section reviews test methods used to measure short-term FI and body composition in children.

2.1.1. Diagnosis of Obesity

Body Mass Index (BMI) is the most widely used method to assess body weight status in children, and is calculated as body weight in kilograms (kg) divided by height in meters squared. In adults, normal weight (NW) is defined as a BMI between 18.5 to 24.9, overweight (OW) as 25.0 to 29.9, and obese (OB) as a BMI of 30.0 or higher [8]. Age and sex specific BMI growth charts have been developed by the Centers for Disease Control (CDC) in the USA for use in the pediatric population and adopted for use in Canada [9]. The CDC growth charts are recommended to determine body weight status in 2-18 year olds. NW is defined by a BMI percentile that is between the 5th and 85th percentile for age and gender, OW if the BMI falls between the 85th and 95th percentile, and OB is defined when BMI percentile is equal to or above the 95th percentile.

2.1.2. Prevalence of Obesity

In Canada, data from the 2004 Canadian Community Health Survey showed that 18% of children aged 2-17 were OW, and 9% were classified as OB [10]. This represents an obesity rate that is 3 times higher than data from the Canadian Community Health Survey in 1978/79 data.
when only 12% of children in this age range were OW, and 3% OB [10]. The estimated economic burden of obesity in Canada has increased from $3.9 to $4.6 billion between 2000-2008 [11]. In 2006 total direct costs of obesity and its co-morbidities were $6.0 billion, with 4.1% of total health expenditures in Canada attributed to overweight and obesity [12].

2.1.3. Consequences of Obesity

Obesity persists into adulthood in approximately 50% of OW children and adolescents [13], resulting in increased risk of chronic diseases in later life. Co-morbidities associated with childhood obesity impact on physical, physiological, and psychological well being. Overweight and obese children are at higher risk of developing conditions that were previously more commonly observed in late adulthood [13]. In a sample of 294 overweight and obese children and youth between 3-19 years old, 51% had abnormal cholesterol levels including high low density lipoprotein (LDL) and triglyceride levels[14]. In addition, Type II diabetes in children represents up to 50% of new cases [15] consisting of glucose intolerance and insulin resistance [16].

Psychological problems associated with childhood obesity include poor self-esteem, depression, anxiety, and feelings of rejection [17]. Compared with normal weight children, obese children have lower self-perceptions and report lower ratings of self-worth than NW children [18].

2.1.4. Causes of Obesity

Childhood obesity is complex with many interacting factors influencing energy balance including biological and environmental factors. Significant effects of genetic factors on body weight (BW) have been evaluated through the study of monozygic twins. Twin studies have
shown that between 20% to 60% of the variation in BMI can be explained by genetic factors [19]. In Swedish identical male twins measured from birth to 18 years of age, heritability of BMI was high and additive genetic variance components were closely correlated to BMI [20]. In Finnish twins an increase in heritability of BMI with age was observed in children and adolescents [21]. Although genetic factors play a significant role in individual susceptibility and development of obesity, it is unlikely that in a span as short as 20 years there has been a change in the frequency of genes and the gene pools have changed significantly and therefore other factors must play a stronger role in the increased prevalence of childhood obesity.

The term obesigenic environment refers to a set of circumstances that encourage people to eat and drink more calories than they expend and to become obese [22]. Some of these factors include increased portion sizes [23], increased consumption of food outside the home, [24] increased sedentary behaviors such as television viewing [25], increased consumption of sugars containing beverages [26], and decreased physical activity [27].

Portion sizes have increased within homes, supermarkets, as well as in restaurants and fast food establishments [23] and this increase has occurred concurrently with the rise in obesity. Food products such as cookies, muffins, and bagels, as well as fast food items such as french fries, hamburgers, and soda have increased 2 to 5 times in size from their original serving sizes [28]. These larger portion sizes lead to significantly increased energy intake in controlled experimental settings. Subjects provided with a lunch meal of 500, 625, 750, and 1000 g portions over 4 weeks of which they could eat ad libitum amounts, consumed 30% higher food intake in the 1000 g portion compared to when offered 500 g portion [29], suggesting larger portion sizes can override physiological mechanisms of FI and energy balance.
Fast food consumption has been hypothesized to contribute to childhood obesity due to its low cost, high availability, and weak effect on appetite although the later has not been tested in children. Consumption of fast food has markedly increased particularly among adolescents over the past two decades [30]. Canadians spend an average of 30% of their food budget on food outside the home [10]. Youth with fast food restaurants in close proximity to their schools have a higher risk of obesity than those with limited access [31]. Children and adolescents who consume fast food have an increase in total energy intake of approximately 63, 132, and 370 kcal per day in children age 4-8, 9-13, and 14-19 respectively compared to non fast food consumers. This accounts for 29-38% of total daily intake [32]. Overweight adolescents in particular illustrate poor compensation for energy from fast food [3], which may contribute to increased weight gain and obesity.

A 30% increase in available sugars in the United States from 1971-1997 has been hypothesized to have contributed to overeating and obesity in children [33]. For several reasons however, the increased availability of sugars as an independent contributor to the obesity epidemic, is uncertain. Concurrent to the increase in sugar availability in the food supply, there has been a proportional increase in per capita availability of poultry (84%), fats and oils (47%), dairy products such as milks (423%) and yogurts (111%), fruit (28%) and vegetables (72%), as well as total energy (15%) [34]. This data, collected in the form of disappearance data, is reported on the basis of per capita potential for consumption; however it is estimated that 30% is wasted or spoiled and not consumed. Additionally, the associations between sugars consumption and OW cited as support for the sugars-obesity hypothesis have been raised from epidemiological and observational studies and therefore are unable to determine a cause and effect relationship.
The increased prevalence of obesity has also occurred concurrently with the increased availability of energy-containing sweeteners [35]. The rise in obesity has been linked to the increased consumption of soft drinks [36, 37], as reported by Ludwig et al, who showed that in children, for each additional serving of sugar-sweetened beverage, BMI increased [37]. These sugar-sweetened drinks are said to promote obesity by virtue of their low satiety and high added sugar content [38], however sugars in solution given to both adults and children have been shown to have effects on subjective satiety and FI at a following meal [7, 35, 39, 40].

Reduced energy expenditure also plays a role in energy balance [27]. Physical activity (PA) levels have declined dramatically with only 7% of Canadian children meeting the current recommended daily PA levels [41]. Sedentary activities such as television viewing (TVV) may contribute to overweight and obesity by decreasing time spent being PA, and TVV has been also been associated with increased energy intake and diminished control over food intake in young boys [25]. Canadian children spend approximately 2.25 hours on weekdays and 3.75 hours on weekends watching television or playing video games [42]. A prospective study on children age 4 to 11 demonstrated the relationship between TVV and body fatness. Children reporting high levels of TVV at 3.0 hours or more per day showed the greatest significant increase in mean sum of skinfold measures than those in the middle (1.75 to 3 hrs/day) or low TVV (<1.75 hrs/day) [43]. Reducing television and videogame usage may therefore be one strategy used in the prevention of childhood obesity [44]. The built environments also play a significant role in PA levels of children, and those lacking access to sidewalks, paths, parks, playgrounds, or recreational centres have 20-40% increased risk of becoming obese than those who do have access to these [45].
2.2. Food Intake Regulation in Children

Young children suppress FI when provided snacks before ad libitum access to meals when they are allowed to focus on internal cues of hunger and satiety [46]. Preschool children 3-5 years of age compensate for calories from caloric sweeteners and fat compared to a non-caloric control [47]. After consumption of high energy, low energy, and no energy preloads, young children 6-7 years adjusted their intakes in direct proportions with the energy content of the preloads, while older children age 8-9 adjusted their energy intakes less well [48]. The younger subjects consumed 12% less after a low energy preload and 22% less after the higher energy preload, while older subjects reduced their intakes by only 13 and 15% after the low energy and high energy preloads respectively [48]. Additionally in 9-10 year old subjects after preloads of sucrose and aspartame children consumed nearly identical amounts of ad libitum lunch [49], providing support to the hypothesis that age differences in caloric consumption (CC) may reflect true developmental differences in responsiveness to energy density [50]. Other studies have found no difference between children age (4-6 years), adults age (18-26 years), and elderly age (61-86 years) subjects after preloads of yogurt with varying energy densities and macronutrient composition [51].

2.3. Effect of Macronutrient Composition on Food Intake Regulation

Mechanisms over FI are sensitive to both energy content and macronutrient composition of food. The three macronutrients, protein, carbohydrate and fat, exert different effects on satiation and satiety independent of their caloric content [52]. The hierarchy of the effect on satiety of macronutrients was observed when adult subjects reported increased fullness and decreased hunger and FI after high protein and carbohydrate compared to high fat preloads [53].
The hierarchy of protein>carbohydrate>fat represents the effect on satiety each of the macronutrients exert. The effect of macronutrients on FI control in children is unknown.

2.3.1. Carbohydrates and short-term food intake regulation

Carbohydrates are a main source of energy for the brain and contribute between 40% to 80% of total energy intake [54]. Sugars suppress FI in both children [39, 50] and adults [35, 55, 56]. In the form of sucrose, glucose, and fructose, carbohydrate preloads or meals suppress appetite ratings and short-term FI by an amount approximating their energy content [7, 39, 57]. Experimental studies have demonstrated that consumption of sugars containing beverages reduce short term FI and energy is compensated for, but this compensation depends on source and type of sugar [58]. These results are in contrast to the hypothesis that sugars by-pass regulatory systems [59], promote FI, and contribute to weight gain and obesity [60].

2.3.1.1. Glucose

Glucose in solution suppresses FI and increases satiety similarly to sucrose in both adults and children. However research has shown varying results. In adults, high concentrations of glucose mixed with fructose resulted in the greatest appetite suppression and lower subjective appetite. The glucose 80%, fructose 20% (G80:F20) preload solution resulted in a significantly higher CC at the next meal at 92%, while other test sugars such as G50:F50 and G20:F80 produce significantly lower CC scores [40] and weaker effects on satiety. Adult males given a glucose preloads of 75 g [55] and 50 g [61] in liquid form reduced FI and lead to almost perfect CC at a test meal one hour later.

The effect of sugars on FI in children is limited by minimal reports. Boys given a treatment of 50 g glucose in 250ml of water decreased FI at a test meal 60 minutes later when
compared to a non-caloric control [25]. Both NW and OW boys also significantly reduced their FI after glucose preloads, with total energy intake including preload and ad libitum pizza lunch not differing from that of a control [7].

2.3.1.2. Sucrose

The effect of sucrose on the suppression of FI has been reported from experimental studies that provide preload solutions of sucrose, followed by lunch test meals after fixed delay intervals. In adults, preloads of 20 g and 40 g did not result in a significant reduction of intake at the following meal [62]. Adult males 18-35y energy intake at a meal 60 minutes following a sucrose preload of 75g was significantly lower than that of a sucralose control [55] and a sucrose preload of 300 kcal significantly suppressed FI at a test meal 80 minutes thereafter more than did the sweet control [40] with a CC score of 89%.

Young children suppress FI at a test meal after the consumption of sucrose in solution but the effect depends on the size of the treatment dose and time to next meal [35, 49]. It was found that 9-10 year-old children in the NW range reduced FI at a test meal for the calories consumed 30 min earlier in the form of sugar-containing drinks, but reduced intake primarily from sweet foods provided in the buffet meal, perhaps in part, as a result of sensory-specific satiety [63, 64]. When the preload of sugar was given 90 minutes before the meal, it had no effect on lunch-time FI or food selection, resulting in an increase in total energy intake, demonstrating that the acute appetite effect of a caloric preload is time dependent [35]. It was also found in recent studies in boys as well as in young men in the normal body weight range demonstrate excellent compensation for the energy content of sugars [56].
2.3.1.3. High-fructose Corn Syrup

High-fructose corn syrup (HFCS) is a mixture of fructose and glucose, found most commonly in the concentrations of 42% fructose, 58% glucose in food products, and 55% fructose, 45% glucose in beverages [65]. Recently replacing sucrose as the most common caloric sweetener, due to its low manufacturing costs and higher sweetness, HFCS now represents 40% of all added caloric sweeteners in the United States [66]. In Canada, data on the availability of HFCS in the food supply is not available however the main use is in sweetened beverages. Indirect estimates of HFCS consumption are 2% of daily caloric intake for females and 3% for males [67].

It has been hypothesized that the replacement of sucrose with HFCS from sucrose has contributed to the increased prevalence of obesity. Solutions of differing glucose to fructose ratios were compared including HFCS (G45:F55), sucrose, G20:F80, and G80:F20 [40]. HFCS lead to a reduction of FI at the next meal which did not differ from the FI observed after sucrose or glucose preloads [40]. Similarly no differences on subjective satiety or FI were observed after preloads of sucrose, milk, cola, or HFCS 55 and HFCS 42 [68]. These results demonstrate that HFCS had similar effects to other equicaloric sugars solutions on FI [69], and does not support the hypothesis that the replacement of sucrose with HFCS in the food supply is a contributing factor to obesity because of differences in their short-term physiologic effects [40, 66].

2.3.2. Protein and Food Intake Regulation

Proteins vary in their effect on satiety as both type and amount of protein have an impact on satiety, subjective appetite, and FI at next meal [70-73]. A comparison of egg albumen, whey, and soy protein in 0.65 g/kg body weight dose, resulted in a decrease in FI at the test meal 60 minutes later after both soy and whey protein and cumulative energy intake after both soy and
whey protein, but not egg albumin, treatments were lower than that of the control [71]. A further comparison of egg albumin, casein, gelatin, soy protein, pea protein, and wheat gluten differed in its results, reporting no significant variation in hunger or satiety feelings, plasma glucose, or insulin responses between test meals [72]. The latter measured the effect of protein source in a mixed meal to test satiety over a longer time frame of 8 hours [72], which may partially explain the variation in results.

Support for a greater effect of protein on short-term FI compared to carbohydrate [70, 74, 75] or fat [76] was illustrated in children by Araya et al [77]. Children 5-6 y had significantly higher energy intake and greater satiety at a high carbohydrate meal than at a high protein meal. Protein was also the only macronutrient to have an effect on short-term satiety and reduce FI in adult females [78] and a reduction in food consumption and subjective hunger is observed after consumption of a high protein beverage enriched with whey protein isolate [75]. Meals higher in protein providing at least 20% of energy [79] have been shown to increase satiety more than meals lower in protein [80, 81]. Significantly higher satiety [81] and subsequent reduction in FI [82] has been reported for meals higher in protein when compared to adequate protein [81] or high carbohydrate meals [82].

2.3.2.1. Cow's Milk

There are several components in dairy foods that are hypothesized to contribute to satiety and suppression of FI, including casein, whey, active peptides, and glycomacropeptide (GMP). Milk products have been hypothesized to have an antiobesity effect [83] with both observational [84] and experimental studies [85] providing support for this hypothesis; however research has been inconclusive in determining the mechanisms of by which the health benefits of dairy promote healthier body weights [83]. It is suggested that milk is more satiating than cola or juice
by way of its higher protein content, however the results of studies comparing milk to sugars containing and control beverages, have also been varied and limited [69, 86]. For example, a comparison of orange juice, low fat milk, cola, and sparkling water in young adults reported no differences in food consumption at next meal 2.25 hours later after consumption of a 1036 KJ portion with energy intakes being the same after all energy containing beverages [87] indicating that milk did not suppress FI more than any other test beverage. Similarly additional research shows no differences on FI after milk, sucrose, HFCS, fruit drink, and orange juice preloads [69, 86]. Sucrose, high-fructose corn syrup, and milk led to similar reductions in FI suggesting that energy not composition is the determinant of FI [69]. More recently however Dove et al compared a 600 ml dose of skim milk to a sugar containing fruit drink at breakfast, where milk resulted in higher self reported ratings of satiety, and a decrease in FI at the next meal 4 hours later than did the fruit drink [6] which may have been related to the protein content in the milk.

There are two types of protein found in cow’s milk. Whey protein contributes approximately 20%, while casein comprises the remaining 80% of the protein in milk [88]. In the 1990’s Boirie et al. developed the concept of “fast” and “slow” proteins to represent the speed at which proteins were digested and absorbed, with whey representing the former and casein representing the later [89]. The slower digestion of casein is attributed to the coagulation is undergoes in the stomach after interaction with the gastric acid [90]. This slower rate of gastric emptying results in slower rises and lower concentrations of plasma amino acids, but these levels are sustained for a much longer period of time [91]. When compared to each other, whey protein has been shown to have a larger effect on short term satiety and FI, [73, 92] however whether or not casein would exert a greater effect in the long term, has not been thoroughly researched. It is suggested that the two proteins work to control both short and longer term satiety [91].
2.4. Effect of Time to Next Meal on Food Intake

Time to the next meal is a factor in studies of short-term FI. Adults have shown partial or complete compensation for calories when time to next meal was 20 [93], 30 [86, 94], 50[69], 60 [56, 94, 95], and 80 [40] minutes. Less research is available for children however compensation has also been observed in younger children using 20-30 [96, 97], 60[39, 98], and 90 minutes [47]. Little research has been completed in older children, but children age 9 to 10 compensated for sucrose preloads at a meal 30 but not 90 minutes later [49]. A delay of 60 min or longer is commonly used in research comparing the effect of carbohydrates and whey protein on short-term FI in adults, and research in boys age 9-14 years yielded results consistent with that of adults [7].

2.5. Physiological Control of Food Intake

Food intake is governed by physiological mechanisms and control involves both short and long term physiological inputs to regulatory centres in the brain. The initiation and termination of feeding is regulated by a number of hormones which interact with the central nervous system. These hormones create the sensations of hunger and fullness. FI control mechanisms are sensitive to both the energy content and macronutrient composition of food.

2.5.1. Short-Term Gastrointestinal Hormones

Hundreds of anorexigenic and orexigenic short-term gastrointestinal hormones exist in the body and are known to have an effect on satiety and short term FI. The release of these hormones into the gastrointestinal tract occurs in response to FI and is affected by macronutrient composition of a meal. Carbohydrates, protein, and fat, each suppress FI by different mechanisms and involvement of the release of differing satiety hormones.
Carbohydrates have been hypothesized to promote satiety through their effect on glucose [99] and insulin secretion [100]. Compensation for sucrose is moderately higher in comparison to lower glycemic index starches amylose or amylopection and a greater reduction in FI by the preloads providing a greater glycemic response supports the hypothesis that there is an inverse association between blood glucose and subsequent short term FI [55]. Other satiety hormones observed to be involved in carbohydrate induced satiety include Glucogon-Like Peptide 1 (GLP-1) [101] and Cholecytokinin (CCK) [102].

Of the macronutrients protein, carbohydrate, and fat, protein exerts the strongest effect on satiety which is partially attributed to its effects on satiety hormones [73]. When compared to glucose, soy, and gluten, whey protein preloads suppressed ghrelin, a hunger inducing hormone, and elevated levels of CCK and GLP-1 [101]. The satiety hormone Peptide Tyrosine Tyrosine (PYY) release is also stimulated by meals high in protein [103]. The effect of fat on satiety has not been explored in children, but in adults fat has been observed to increase the release of CCK [104]. Short-term gastrointestinal hormones will be further discussed below.

2.5.1.2. Cholecystokinin

Cholecystokinin (CCK) is secreted from the duodenum in response to FI and acts to stimulate pancreatic and gall bladder enzyme release, inhibit gastric emptying, increase intestinal motility [105], and has been documented to reduce meal size [106] and acts to suppress appetite. Administration of CCK-A receptor antagonists leads to a reversal of FI suppression in rats after both carbohydrate and fat sources [102], although this effect is dependent on both the composition of the fat or carbohydrate, and the timing of administration. Results from human studies have demonstrated weaker associations with CCK-A receptor antagonist Loxiglumide
with administration increasing FI insignificantly [107], however infusion of CCK significantly decreased FI [106] and increased CCK response increased subjective satiety [108].

Macronutrient composition determines the amount of CCK release. For example, hydrolysis of fat in the small intestine has been related to reduced meal size and caloric consumption which is paralleled by increased plasma concentrations of CCK [109, 110]. Consumption of meals containing dairy have also been associated with higher stimulation of postprandial CCK release [111]. In animal studies, although CCK was observed to decrease meal size, meal frequency increased [112], and with continuous or repeated infusion tolerances develop [113]. Additionally CCK has a short half-life and administration is ineffective at suppressing FI if given more than 15 minutes prior to feeding [114]. It is possible that a synergistic effect exists between CCK and other gut hormones and may need to be used in combination with other satiety signals to provide benefits in weight reduction [106], this will need to be tested.

2.5.1.3. GLP-1

GLP-1 is inversely correlated with body mass and acts as a FI inhibitor [105]. Due to its short half life, as GLP-1 is broken down rapidly, and although it is effective in short term FI regulation, GLP-1 has not been shown to have effects on longer term energy intake. When administered through peripheral injection the satiety hormone dose-dependently decreases FI in human subjects in both obese and non obese subjects [115], demonstrating that OB individuals are still sensitive to its’ effects, but their lower circulating GLP-1 levels may support their continued obesity [105].
GLP-1 release is also influenced differently by macronutrient composition. Using oral
fructose and glucose Kong et al tested the effect of these sugars on plasma GLP-1
concentrations, and observed that oral glucose had a bigger effect on its’ release [116]. GLP-1
secretion was prolonged after liquid preloads of soy, whey, and gluten when compared to a
glucose treatment which suggests that the satiety hormone may also contribute to the satiety
associated with dietary proteins [101].

GLP-1 is an incretin hormone, which is a type of hormone secreted from the gut that
augments insulin secretion. It’s insulinotropic effects are dependent upon glucose, requiring a
certain amount of glucose to be present for GLP-1 to have an effect on insulin secretion [117].

2.5.1.4. Peptide Tyrosine Tyrosine (PYY)

Peptide YY is released from the gastrointestinal tract and is correlated with energy intake
[105]. Although research on its’ effects is limited, results have demonstrated the plasma
concentrations of PYY rise rapidly after FI and remain high for several hours thereafter [118].
Infusions of PYY have been shown to reduce both FI and subjective appetite ratings [119]
demonstrating that this hormone may be an important satiety signal.

2.5.1.5. Ghrelin

Ghrelin is produced in the stomach and is the only peripheral orexigenic satiety hormone.
Circulating ghrelin levels are greatest during fasting and decrease following FI [120], suggesting
that meal initiation may be signaled by a rise in preprandial ghrelin levels. Intravenous doses of
ghrelin have been shown to cause significant increases in FI at buffet meals when compared to
saline [121] providing evidence of the appetite stimulating effect of this hormone. While
individuals suffering from anorexia nervosa or those who have recently lost weight [122] display
increased fasting ghrelin levels, OB individuals have been shown to have reduced plasma ghrelin levels, compared to NW individuals [123], which may be increased after weight loss occurs [105]. BMI has been implicated by many research studies to have a negative correlation with circulating ghrelin levels [123-125], although research in both children [126] and adults [121] have found this relationship to be insignificant when BMI ranges of OW and NW subjects are less extreme. Insulin resistance in both adults [123, 127] and children [126] has also been correlated with fasting ghrelin levels, but further research is needed to understand the mechanisms of this relationship.

2.5.2. Long-Term Regulation of Energy Balance

Long term regulation of energy balance is partially maintained through additional gastrointestinal hormones such as insulin and leptin. Unlike the short-term regulating hormones, dramatic postprandial increases or decreases in circulating insulin and leptin levels do not occur [128]. These hormones are referred to as adiposity signals, due to the relationship between circulating hormone and body fat levels [100]. Changes to the plasma levels of the adiposity hormones indicate an altered state of energy homeostasis, and in response the brain reacts to restore body fat to regulated levels by adjusting energy intake and utilization [129]

2.5.2.1. Leptin

Leptin is synthesized by adipose tissue, and provides information to the hypothalamus regarding body fat stores [128]. Leptin functions in a negative feedback loop to regulate medium and long-term energy balance as opposed to functioning as a short-term satiety signal [130]. Circulating leptin concentrations change in response to both short-term reductions in energy intake as observed in low caloric diets [131] or fasting and re-feeding, as well as in response to long term decreases in fat stores [132]. While serum leptin is positively correlated with body fat,
BW, hip circumference, and BMI, body fat has been identified as the strongest predictor of serum leptin levels, that is individuals with higher body fat percentage have higher serum leptin [131]. Decreased leptin levels are associated with increased hunger and appetite, and therefore decreases in serum leptin which follow the reduction of FI and adiposity may also contribute to the weight regain most common to dieters [133].

2.5.2.2. Glucose and Insulin

The effect of insulin on long term FI regulation is based upon its relationship to circulating blood glucose levels. The glucostatic theory hypothesizes that FI occurs in the presence of low blood glucose. It was theorized that when the brain detected a low utilization of glucose, it would send stimulus for meal initiation, while times of high glucose utilization would lead to the onset of satiety [99]. Tests of hypothesis centered around the glucostatic theory have demonstrated only a weak relationship between blood glucose concentrations and appetite [128].

Additional research related to blood glucose concentrations and FI regulation have made use of the glycemic index (GI), which categorizes foods based on the subsequent rise in blood glucose concentration they cause. Contradictory to the glucostatic theory, this more recent theory proposes that foods of high GI which cause a more dramatic rise in circulating blood glucose levels result in high insulin responses, followed by more sudden drops in glucose levels, which would consequently lead to hunger [59, 134]. Research by Flint et al showed no effect of glycemic response on subsequent FI at next meal, disputing the hypothesis that appetite sensations and FI are determined by glycemic responses [135]. This was observed after healthy young men consume 10 breakfast combinations each consisting of 50 g carbohydrates, all varying in GI scores, following which lunch intakes were measured [135]. A 2007 meta-analysis of the relevant research studies supports this outcome concluding that insulin, but not glucose,
might be the more important satiety signal in short term appetite regulation [136]. In addition glucose, with the highest GI, along with other high GI carbohydrates polyose and sucrose resulted in lower meal time intake than did lower GI carbohydrate [55] providing evidence that FI and subjective appetite are inversely associated with blood glucose concentrations.

Research testing circulating blood glucose and insulin levels simultaneously has provided evidence that insulin response, though correlated with glucose levels, is not solely dependent on the glycemic response. While insulin release is related to the amount of plasma glucose this only represents as little as 23% of the variation in the insulin response [137]. As an adiposity signal, insulin has been shown to play a crucial role in long term FI regulation, energy homeostasis, and BW [129]. When changes in plasma insulin levels are detected by the central nervous system, the brain adjusts FI to restore the body to normal levels [129] which acts to decrease FI [100] and increase energy expenditure [138].

2.6. Short-term Food Intake Regulation in Obesity

The effect of obesity on short-term FI regulation has not been reported extensively in the research literature but may be a factor in FI regulation [139]. NW individuals may have better short-term FI regulation than OW/OB individuals, which has been observed in boys. OW/OB subjects failed to reduced their FI after a 50 g whey preload, compensating for only 39% of the energy provided from the whey while normal weight subjects demonstrated significantly higher CC at 91% [7].

Short and long term satiety hormones previously discussed are one mechanism which may differ in NW and OB individuals. The relationship between concentrations of these hormones and appetite may be complicated by the presence of excess weight. Individuals who are OW and OB often show signs of insulin resistance, a factor which may be associated with
dysfunctional appetite regulation. Changes in baseline ghrelin after feeding for NW but not OB
subjects have also been reported [140], which demonstrates possible evidence that OB
individuals may have impaired suppression of the drive to eat. Obese subjects also have been
shown to have significantly lower fasting concentrations of PYY than that of the NW individuals
and have a deficiency of secretion of postprandial PYY [119] which may contribute to the
maintenance of obesity [105].

In children these differences in short-term FI based of weight status are also observed,
but research is limited. Fat mass (FM) has been inversely associated with CC of carbohydrates in
girls but not boys age 3- to 5-y [139]. Obese individuals have also been reported to experience
high levels of hunger and low levels of control over eating [141] and higher degrees of restrained
eating [142] which will be discussed in the next section.

2.7. Short-term Food Intake in Relation to Sex

Sex differences have an effect on control of FI. Several mechanisms have been identified
as contributors to these differences including restrained eating and differences in the release and
response to satiety hormones which impact hunger and satiety signals. In order to understand the
differences between male female regulations of FI these factors must be considered.

High cognitive restraint has been correlated with higher BW [143]. Restrained eaters
experience high levels of hunger and low control over their eating [142], which may lead to
binges associated with increased BW. Females demonstrate higher levels of emotional and
restrained eating compared to males [142], which may contribute to differences observed
between sexes. Girls as young as 5 y report restrained, emotional, and disinhibited eating [144].
This restrictive eating in young girls is associated with higher snack intake and adiposity [145].
Restrained eating may also affect sensitivity to satiety hormones as a study by Burton-Freeman described. Females characterized as restrained eaters consumed greater amounts of energy than their non-restrained counterparts, and CCK response in these restrained subjects was diminished [146]. This effect is also seen in patients suffering from bulimia nervosa [147], suggesting a change in physiological regulation.

A sex difference in regulation is suggested by females demonstrating lower CC for energy than males. This result was reported by Rawana et al. who found after a milk preload females and males compensated by 85% and 100% respectively [95]. This is in agreement with research by Davy et al. who reported FI regulation to be significantly less accurate in females than in males after a preload study using yogurt [148]. In this example female subjects demonstrated a 12.5% lower compensation score than their male counterparts [148].

In animal studies sex differences after a 24 hour fast resulted in a significantly greater increase in FI by female rats than males after the first day of fasting, and overall higher net energy intake after a 3-day study period [149]. In the female rats fasting induced higher ghrelin levels, and reduced plasma leptin levels which may indicate the females are more sensitive to fasting and more likely to overeat after a short-term energy deficits [149]. Human research by Greenman et al. also showed female subjects had significantly higher ghrelin levels than males after both glucose and lipid loads as well as in the fasting state [124].

2.8. Measurement of Food Intake and Body Composition in Children

In the following section test methods and instruments used in measurement of short-term FI and BW will be described. These test instruments have been demonstrated to be reproducible and valid however there is limited literature in pediatric populations.
2.8.1. *Measurement of Short-Term Food Intake*

The most common method for assessing short-term FI is through the use of a preload design. In this approach subjects consumed a control, none caloric treatment, or a fixed amount of a caloric test treatment, followed by a test meal at a fixed time point thereafter where they may eat as much or as little as they desire [50]. Caloric compensation for the test treatment is then calculated to assess the effect of the treatment by comparing the difference of the mean lunch intake in the no calorie preload condition and the mean lunch intake for the caloric preload divided by the energy of the preload, multiplied by 100 [51]. A CC score of 100% representing a subject reducing her lunch intake by 175 kcal when given a calorie-containing (175 kcal) preload (treatment) compared with days when the subject was given a control preload. CC scores of less than 100% indicate failure to fully compensate at the test meal, whereas scores above 100% indicate that the girls are overcompensating by consuming less energy at the test meal than that contained in the preload.

The preload design has been used to describe the effects of carbohydrates, fat and protein preloads on short-term FI suppression in both men and women. Fewer studies have been completed in children, however this method has been shown to be reproducible in this population. In boys, lunch-time food and water intake after a glucose preload was similar on two separate occasions 1 week apart with a meal energy intake of 925 ±139 kcal and 988 ± 147 on the two respective days, representing excellent reproducibility [150].

2.8.2. *Hunger, Satiety and Satiation*

Hunger, satiety and satiation are terms which are frequently used when discussing food intake regulation studies. As part of the body’s appetite control system, hunger is a biological drive which impels individuals to search for food, while satiety and satiation are involved in
limiting the intake of energy. Hunger is responsible for determining what, how much, and when to eat [151]. After energy consumption has begun physiological processes occur which inhibit further consumption. This process which causes the discontinuation of feeding is referred to as satiation, or intra-meal satiety [152] and is often assessed by volume, weight, energy, and macronutrient composition of the food eaten. The further suppression of food consumption between meals due to the continued feeling of fullness after eating has ended is referred to as satiety or inter-meal satiety [49]. It may be assessed in terms of the duration of the suppression of hunger. Taken together, the mediating processes (sensory, cognitive, post-ingestive, and post-absorptive) involved in satiation and satiety is often referred to as the satiety cascade.

2.8.3. Subjective Appetite

Visual analogue scales (VAS) have been used previously to measure subjective appetite in young children following caloric and non-caloric preloads [7, 150, 153]. The original method developed by Hill and Blundell (1982) [154] comprised 6 questions: ‘How strong is your desire to eat?’ (very weak/very strong), addresses an emotional desire for food, ‘How hungry do you feel?’ (not at all hungry/as hungry as I’ve ever felt), addresses the physical need for food; ‘How full do you feel?’ (not at all full/as full as I’ve ever felt); ‘How much do you think you could eat? (nothing at all/a large amount); ‘Urge to eat’ (no urge to eat/strong want to eat now, waiting is very uncomfortable); ‘Preoccupation with thoughts of food’ (no thoughts of food/very preoccupied difficult to concentrate on other things).

The predictive validity of VAS is a matter of debate in the literature [55, 155]. Research has supported the reproducibility of the VAS to measure appetite ratings in studies measuring short-term FI. Subjective appetite has been observed to decrease following macronutrient preloads and is positively correlated with measured FI [156, 157]. In a study in adult males,
individual appetite scores measured before lunch of 5 different occasions were similar [158],
additionally the coefficient of repeatability (CR) has also been calculated to show the 
reproducibility of VAS scores to be low after male subjects were given identical meals [159].
The result was a low CR, which suggests the scores are reproducible [159].

VAS has been utilized to measure short-term FI in several research studies in both adults [40, 74, 160] and children [25, 161]. VAS are reproducible in children as was demonstrated by Bellissimo et al. (2008). For example, when boys consumed a 50g glucose preload treatment on two separate weekend mornings both subjective appetite and FI were reproducible. Although subjective appetite scores were highly variable between days, when change from baseline scores were analyzed scores did not differ between test days [150]. Also in boys age 9-14 years average appetite scores calculated from VAS were positively correlated with FI in both NW and OB subjects [7] which suggests that children understand the scales and complete the VAS in a manner that is reflective of their feeling of appetite [7].

2.10. Measurement of Body Composition

Accurate assessment of body composition is important in many areas of obesity and
nutrition-related research. Some of the more frequently used body composition techniques to estimate fat-mass (FM) or fat-free mass (FFM) in children are described below.

2.10.1. Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) is a method used to measure body composition using dual x-ray beams each with different energy levels, which allow for the differentiation between tissues with different densities [162]. The accuracy of the measurement of body composition in children using DXA have often compared DXA against total carcass chemical analysis in pigs [163, 164]. The precision of the DXA measurements was determined through
repeated scans of 4 pigs with representative weights similar to that of children and showed reproducibility of 1-2% [165]. Body weight was found to be highly correlated with direct measure of weight, however the division of mass between the bone mineral, fat, and lean tissue was found to be over or underestimated when using the adult scan mode [165]. A more recent study of 18 pigs within the pediatric BW range found that lean and fat mass were highly positively correlated with the reference method ($r = 0.98$), but duplicate scans were significantly different which suggests that correction factors should be used to improve the accuracy of the scan in children.

Advantages for the measurement of body composition in children through the use of DXA is its short time duration (<10 minutes), high precision and low dose of radiation (1 mSv or 1/100th of the equivalent radiation exposure of a chest x-ray) [166].

2.10.2. Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) is a noninvasive body fat measurement technique that is used to measure body fat composition in both adults and children [167]. It is a relatively simple and inexpensive test that is based on the measurement of an electric current which is conducted through the cells of the body. Fat free mass (FFM) which has a greater amount of water and electrolytes conducts the electric current better than fat mass which does not [168]. A greater amount of FFM in the body will therefore result in a lower resistance to the flow of the current. In order to calculate the amount of FM a prediction equation must be used. In young children the Kushner equation [169] is recommended for estimating total body water from $\text{height}^2$/resistance and has been cross-validated [170].
Bioelectrical analysis has been cross-validated with DXA [171] and anthropometry [172] in adults and against total body water [170], total body potassium [173], and DXA [174] in children. Correlations between BIA and DXA were 0.88 for a healthy population sample of 591 subjects. BIA was however observed to overestimate body fat in lean subjects and underestimate for obese subjects [175]. Similarly results of Eisenkolbl et al. (2001) found BIA measurements of body fat mass to be significantly lower than DXA [176], and when compared to hydrodensitometry for the measurement of weight loss, BIA underestimated loss of FFM [177].

A limitation of BIA measurements lies in the required knowledge of hydration status as bioelectrical resistance provides an estimation of total body water which is then transformed into fat-free mass. Although constant in adults, hydration status is known to vary in children [170, 178].

2.10.3. Skinfolds

The skinfold thickness technique is performed by pinching the skin between the thumb and forefinger at specific locations on the body, and placing calipers on the fold. The thickness of the fold is a measure of the fat underneath the skin also known as subcutaneous adipose tissue. This subcutaneous fat correlates with total body FM, which provides rationale for the calculation of body fat percentage from a regression equation of skinfold thickness on total body density [179] [180].

Skinfold prediction equations have been developed for use within a pediatric population [181, 182]. Because skinfold equations lack cross-validity the sample in which the prediction question is to be used should be validated before its use to ensure accuracy. In 98 boys and girls when compared to DXA the Slaughter equation overestimated FM, and Janz et al failed to
validate the Slaughter equation based on the criterion method (Lohman's Siri age-adjusted body density equation) in children [174].

2.11. Summary

The increase in childhood obesity over the past several decades has occurred at an alarming rate. In addition to being at increased risk for obesity in adulthood, obese children are at risk of developing obesity related diseases such hypercholesterolemia, hypertension, and type 2 diabetes in childhood. Environmental factors have been the predominant focus of research on the determinants of obesity, however little research has determined the effect of macronutrient composition and BW on FI, especially in young girls. These factors have the potential to effect physiological regulation of FI, and a greater understanding of their role in FI regulation may help in the prevention of obesity. In children these factors are widely unexplored and therefore the objective of this research was to examine the effect of some of these factors including body composition and macronutrient composition on FI regulation in girls 9-14 years of age.
Chapter 3. Hypothesis & Objectives
3.1. Hypothesis

1% chocolate milk will suppress short-term FI to a greater extent than other sugars containing beverages but the effect will be diminished in OW/OB girls.

3.2. Objective

To determine the effect of isovolumetric drinks of (350ml) 1% chocolate milk, fruit drink, and cola 60 min before a pizza meal on subjective appetite, and short-term FI compared to a water control, in NW and OW/OB 9-14 year old girls.
Chapter 4. Methods
4.1. Participants and Screening Process

Twelve NW (between the 15th and 85th BMI percentile for age and gender), and 11 OW (above the 85th BMI percentile for age and gender) or OB (above the 95th BMI percentile for age and gender) girls were included based on the Centers for Disease Control growth charts [183]. Participant recruitment was done through the use of advertisements in local a local newspaper, the placement of recruitment flyers throughout the community, and through word of mouth. In order to participate, girls had to have been born at full term and normal weight. Children dieting, taking medication, and with any significant learning, behavioural, or emotional difficulties were excluded [7, 25, 150]. Parents who volunteered their children completed, by telephone, a semistructured interview designed to elicit information about the inclusion and exclusion criteria. If a child met study requirements, a screening session was held in the Department of Applied Human Nutrition, where informed consent from the parent and a written assent from the child was obtained. The child’s height (m) and weight (kg) measurements and triceps, biceps, suprailial and subscapular skinfold thickness (mm) using a Harpenden skinfold caliper, were measured and recorded to the nearest 0.1 mm. The sum of 4 skinfold measurements was then used to estimate percent fat mass from a sex specific regression equation [164]. The parent and child were given a tour of the facility to familiarize the child with the facility and the VAS questionnaire to minimize apprehension during the first test visit.

4.2. Experimental procedure:

Subjects arrived at the Evaristus building at Mount Saint Vincent University 2 h after the consumption of a standard breakfast (Table 4.1.) of fat-free skim milk (Baxter’s 1% M.F. Chocolate Milk®, 250ml), Honey Nut Cheerios® (26g, 103 kcal purchased at Sobeys®, Halifax, NS.), and Tropicana Orange Juice® (236 ml, 110 kcal purchased at Sobeys®, Halifax, NS) at
home. The session time was kept consistent throughout the 4 visits (e.g always 10:00am
11:00am, or 12:00pm). Upon arrival subjects were asked if they consumed the entire breakfast, if
any other foods were consumed 10-12h prior to arrival, and if they were taking any medication.
If they reported significant deviations from their usual patterns, they were asked to reschedule.
Subjects then completed a baseline Visual Analogue Scale (VAS) questionnaire rating their
motivation to eat and physical comfort. This questionnaire was again filled out at 15, 30, 45, and
60 minutes thereafter, as well as after the pizza meal at 90 minutes. VAS was also filled out after
consumption of the preload to measure pleasantness and sweetness, and after the pizza lunch to
assess palatability of the meal.

Preload treatments were given in a random order to participants each week. Three sugar
containing beverages were cola (Coca Cola®, 350 ml, 158 kcal purchased at Sobeys®), fruit drink
(Fruite®, 350 ml, 154 kcal), and 1% Chocolate Milk (Baxter’s® 1% M.F Chocolate Milk, 350 ml,
224 kcal) or a water control (Nestle Pure Life® 350 ml, 0kcal). Test beverages were prepared the
morning of the test session a few minutes prior to subjects’ arrival. The subjects were escorted to
a feeding room with individual cubicles to minimize distraction, and served their test beverage.
Subjects completed the beverage within 5 minutes and were given 100 ml of plain water given to
minimize aftertaste. Test beverages were served in opaque cups with lids and straws.

Following the preload consumption, subjects participated in low activity, age appropriate
activities for 60 min, and filled out the VAS questionnaire at 15, 30, 45, and 60 minutes at which
time they were escorted to the feeding room and served an ad libitum pizza lunch. Two varieties
of Deep ‘N Delicious 5” diameter pizza were offered; pepperoni and three-cheese pizzas
(donated by McCain Canada Ltd., Florenceville, NB). Pepperoni pizza (87 g) contains 9 g
protein, 6 g of fat, and 23 g of carbohydrates for a total energy content of 180 kcal. Three-cheese
pizza (81 g) contains 9 g of protein, 6 g of fat, and 22 g of carbohydrate for a total energy content of 180 kcal. At screening children were asked to indicate which type of pizza they would prefer. A fresh tray of pizza was prepared, weighed, and cut into four equal pieces before serving, and the amount left after the meal was subtracted from the initial weight to provide a measure of FI. Fresh trays were provided to the subjects at 60, 70, and 80 minutes. The subjects were instructed to eat until they were “comfortably full” and were provided with a bottle of water (500 ml) to accompany their lunch as previously described [7, 25, 150, 160, 184, 185].
Table 4.1. Nutritional composition of standardized breakfast

<table>
<thead>
<tr>
<th></th>
<th>Honey Nut Cheerios® (26 g)</th>
<th>Baxter’s® Skim Milk (250 ml, 250 g)</th>
<th>Tropicana® Orange Juice (236 ml, 252 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>103</td>
<td>90</td>
<td>110</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>0</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>149</td>
<td>125</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>20</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>1.9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>8.4</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.9</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>
4.2.1. Treatments

The treatments were cola (Coca Cola®, purchased at Sobeys®, Halifax, NS), fruit drink (Fruite®, purchased at Superstore®, Halifax, NS) and 1% chocolate milk (Baxter’s® 1% M.F Chocolate Milk, purchased at Sobeys®, Halifax, NS) and a water control (Nestle Pure Life®, purchased at Sobeys®, Halifax, NS) (Table 4.2). All drinks were provided in 350 ml servings rather than matched for energy content in order to observe the effect of these treatments in their regularly consumed portion size, as 350 ml is a common commercially available portion size of soft drinks. All drinks were served chilled in a covered opaque cup. Subjects consumed their drinks within 5 minutes which was then followed by 100 ml of water to minimize aftertaste. The pH of each treatment is reported as taking the average of three measurements which were obtained using a Fisher Scientific accumet® AB15 Basic and Biobasic pH/mV/ °C. All treatments were 6.5 °C at the time of the measurement. Analytical sugars composition of the cola and fruit drink preload treatments was performed by Maxaam Analytics International Corporation, Mississauga, Ontario using reference method AOAC 980.13 [186].
Table 4.2. Nutritional Composition of Preload Test Treatments

<table>
<thead>
<tr>
<th>Per 350 ml</th>
<th>Water</th>
<th>Fruit Drink</th>
<th>Cola</th>
<th>1% Chocolate Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>0</td>
<td>154</td>
<td>158</td>
<td>224</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>0</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>0</td>
<td>34</td>
<td>38</td>
<td>36</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Sucrose (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>0</td>
<td>18</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>0</td>
<td>53</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>0</td>
<td>16</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Fructose (%)</td>
<td>0</td>
<td>47</td>
<td>58</td>
<td>-</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Glucose:Fructose*</td>
<td>0</td>
<td>1.1</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.00</td>
<td>2.96</td>
<td>2.49</td>
<td>6.77</td>
</tr>
</tbody>
</table>

*Calculated as glucose/fructose.
4.2.2. Test Lunch:

Two varieties of Deep’ N Delicious 5” diameter pizza were used; pepperoni and three-cheese pizzas which were donated by McCain Foods (McCain Canada Ltd.). Pepperoni pizza (87 g) contained 9 g of protein, 6 g of fat, and 23 g of carbohydrates for a total energy content of 180 kcal. Each three-cheese pizza (81 g) contained 9 g of protein, 7 g of fat and 22 g carbohydrate for a total energy content of 180 kcal (Table 4.3.). Cooked pizzas were weighed and cut into four equal pieces before serving, and the amount left after the meal was subtracted from the initial weight to provide a measure of food intake. An advantage of using these pizzas is the lack of crust, which results in a pizza with a more uniform energy content and the elimination of the possibility of subjects eating the denser filling and leaving the outside crust of the pizza. The pizza was served on individual trays at a set time following the preload. Three pizzas were served on each tray (average of 220 kcal/pizza, 49.5% of energy as carbohydrate, 31% as fat and 19% as protein depending on its variety), the first tray was removed after 10 minutes and subjects were provided with a second warm tray, which was repeated again at 20 minutes, giving subjects a total of 30 minutes to consume their lunch. The bottled water served with the pizza was also weighed before and after the test meal to calculate the net amount ingested during the meal.
Table 4.3. Nutritional Composition of test meal pizzas

<table>
<thead>
<tr>
<th>Per 1 Pizza</th>
<th>Pepperoni (87g)</th>
<th>3-Cheese (81g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>400</td>
<td>360</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
4.2.3. Subjective Appetite:

Visual analogue scale (VAS) questionnaires were used to assess appetite at baseline, 15, 30, 45, 60, and 90 minutes, and are composed of four questions or scales. The 100 mm lines are affixed with opposing statements at either end (“very weak” to “very strong”), and subjects mark “X” on the line to depict their feelings at the present moment in time. The questions “how strong is your desire to eat? (very weak/very strong); ‘How hungry do you feel?’ (not at all hungry/as hungry as I’ve ever felt); ‘How full do you feel?’ (not at all full/as full as I’ve ever felt); ‘How much do you think you could eat?’ (nothing at all/a large amount); ‘how thirsty do you feel?’ (not at all thirsty, as thirsty as I’ve ever felt) are asked, and the score was determined by measuring the distance (mm) from the left starting point to the intersection of the “X”. To determine average appetite scores, desire to eat, hunger, and prospective food consumption, and 100-fullness are added, were then divided by four (Appetite score (mm) = [desire to eat + hunger + (100-fullness) + PFC]/4) [7, 25, 150, 160, 184, 185]. Participants physical comfort was also assessed at each other the time points by the question “How well do you feel?” (not well at all, very well).

After participants consumed the preload treatments the sweetness treatment was also measured using the VAS. The question “How sweet have you found the beverage?” was anchored by “not at all sweet” and “extremely sweet”. Acceptance of the drink was also assessed by the VAS question “How pleasant have you found the preload? (not at all pleasant, very pleasant”. To determine the acceptance of the ad libitum pizza lunch the VAS question “How pleasant have you found the food (Not at all pleasant, very pleasant) was filled out by participants at 90 minutes following the lunch meal.
4.2.4. Determination of Dietary Cognitive Restraint

The Dutch Eating Behavior Questionnaire (DEBQ) consists of 33 questions used to assess dietary restraint and disinhibition. It is broken down into three main factors. The first, dietary restraint or cognitive control over eating is measured by the restraint scale which is comprised of the first 10 questions on the DEBQ. Second, emotional disinhibition or loss of control over eating due to emotions is measured as overall emotional disinhibition by questions 11-23, and is then further broken down into (a) specific emotional disinhibition, loss of cognitive control due to specific emotions measured by questions 11, 13, and 15-22, and (b) disuse emotional disinhibition or loss of cognitive control over eating due to diffused emotions measured by questions 12, 14, and 23. The third main factor disinhibition is measured as overall disinhibition by questions 11-33, and as external disinhibition or loss of cognitive control over eating due to the presence of food by specifically by questions 24-33 [187].

The DEBQ has been used previously in nine to twelve year olds to assess differences between obese and non-obese children and has demonstrated significantly higher scores for emotional, external, and restrained eating behavior in obese children [188]. The questionnaire has been tested for validity and has demonstrated adequate psychometric properties in 9-10 year old girls [189] and has also been used to observe the differences between male and female populations in their eating behaviors [187].

4.2.5. Skinfold measurement

Skinfold measurements were determined by use of a Lange skinfold caliper (Beta Technology Incorporated- Cambridge, Maryland). Skinfold measurements were obtained by standard procedures [190], as previously reported [7, 25, 150, 160, 184, 185]. Estimates of body fat were
predicted from regression questions from skinfolds (Cambridge Scientific Industries, Cambridge, MD, USA) [164].

Weight status of each subject was determined through the use of BMI and BMI percentiles as determined through the use of the Centers for Disease Control and Prevention BMI Percentile Calculator for Child and Teen English Version accessed from the website:

http://apps.nccd.cdc.gov/dnpabmi/ [191].

**Equation 1:** Density=1.2063 – 0.0999 * log (sum of skinfold thicknesses at 4 sites)

**Equation 2:** Body fat percentage of body weight= [(4.95/body density) - 4.5) x 100

4.3. Ethical Considerations

Ethical approval for the study was obtained from the Mount Saint Vincent University Research Ethics Board (File Number 2010-101). Parental consent ([Appendix 9.2]) & children’s assent ([Appendix 9.3]) for participation was obtained at the screening session prior to commencing the study. In order to keep all subject names confidential, subjects were given a code number to identify them which was used on all documents and records. Data pertaining to the study was entered into Microsoft Excel files and was only available to investigators. All records relating to participants were kept in a locked cabinet in the Department of Applied Human Nutrition (Evaristus 366) at Mount Saint Vincent University to ensure confidentiality. No disclosure of personal information of the children or parents took place. All documents pertaining to the study will be securely destroyed after a minimum of five years following the completion of the study.
Chapter 5. Statistical Analysis
5.1. Statistical Analysis

All data are reported as mean ± standard error of means (SEMs). Food and water intake, sweetness, pleasantness, and food palatability data were analyzed using a 2-factor Proc Mixed Model procedure SAS (Statistical Analysis System, SAS Institute INC, Carey, NC) with preload treatment (cola, fruit drink, 1% chocolate milk, and water) and group (NW and OW/OB) as main factors. Preload treatment effects on pooled FI data were also analyzed using the Mixed Model procedure with preload treatment as the main factor when 2-factor tests indicated no effect of group. Results were significant at P < 0.05. Post-hoc analysis of statistically significant differences was completed using Tukey-Kramer’s test, adjusted for multiple comparisons when treatment effects or interactions were found to be statistically significant. Caloric compensation between groups was analyzed by Student’s independent sample t-test. Pearson correlation coefficients were used to determine associations among dependent measures and absolute subjective appetite scores including average appetite, desire to eat, hunger, fullness, prospective food consumption, thirst, and preload sweetness, as well as water intake, body composition including body weight, body mass index, fat mass, and fat-free mass, dietary disinhibition, restraint, and emotional eating.

A 3-factor mixed model was used to determine the effect of treatment, group, and time on subjective measures of appetite, thirst, and physical comfort. The pre-meal subjective feelings of appetite are expressed as absolute scores. Change from baseline scores were also reported to control for subjective feelings of appetite upon arrival to test sessions, at 15, 30, 45, and 60 min. This was performed for average appetite (AA) as well as all individual subjective appetite scores. Change from baseline scores adjusted for the energy content of the beverage, were calculated by
dividing the change from baseline scores at each time point (15, 30, 45, 60 minutes) by the energy content of the preload.

Average appetite scores were calculated at each time of measurement for each preload treatment using the formula:

\[
\text{Appetite score (mm)} = \frac{\text{desire to eat} + \text{hunger} + (100-\text{fullness}) + \text{PFC}}{4}
\]

which reflects the 4 questions on the motivation to eat VAS [7, 25, 49, 150, 160, 184, 185, 192].

Caloric compensation was calculated for each subject after each treatment using the formula as reported previously [7, 25, 49, 150, 160, 184, 185, 192]:

\[
\text{Caloric compensation (\%)} = \frac{\text{Control intake (kcal)} - \text{Treatment intake (kcal)}}{\text{kcal in treatment preload}} \times 100.
\]
Chapter 6. Results
6.1. Participants

Twelve NW girls (11.5 ± 0.5) with a BMI of 17.6 ± 0.6 kg/m², BMI percentile 46.8 ± 6.4, and eleven OW/OB girls (12.2 ± 0.4y) with a mean BMI of 24.9 ± 0.8 kg/m², BMI percentile 92.2 ± 1.3, were included in this study and completed all four test sessions (Table 6.1).
Table 6.1. Baseline characteristics of test subjects

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>All Subjects</th>
<th>NW</th>
<th>OW/OB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11.8 ± 0.3</td>
<td>11.5 ± 0.5</td>
<td>12.2 ± 0.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>50.0 ± 3.1</td>
<td>41.8 ± 3.2</td>
<td>58.9 ± 4.3*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.51 ± 0.0</td>
<td>1.47 ± 0.0</td>
<td>1.55 ± 0.0*</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>21.2 ± 0.9</td>
<td>17.6 ± 0.6</td>
<td>24.9 ± 0.8*</td>
</tr>
<tr>
<td>BMI percentile range</td>
<td>68.5 ± 5.8</td>
<td>46.8 ± 6.4</td>
<td>92.2 ± 1.3*</td>
</tr>
<tr>
<td>Fat-Mass (%)</td>
<td>21.5 ± 2.0</td>
<td>17.8 ± 2.1</td>
<td>25.5 ± 3.3*</td>
</tr>
<tr>
<td>Fat-Mass (kg)$^1$</td>
<td>11.7 ± 1.9</td>
<td>7.8 ± 1.2</td>
<td>15.9 ± 3.3*</td>
</tr>
<tr>
<td>Fat-Free Mass (%)</td>
<td>78.5 ± 2.0</td>
<td>82.2 ± 2.1</td>
<td>74.5 ± 2.6*</td>
</tr>
<tr>
<td>Fat-Free Mass (kg)$^1$</td>
<td>38.3 ± 1.8</td>
<td>34.0 ± 2.3</td>
<td>43.0 ± 2.3*</td>
</tr>
<tr>
<td>DEBQ Average Score</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Restraint</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Overall Emotional Disinhibition</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Diffuse Emotional Disinhibition</td>
<td>2.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Specific Emotional Disinhibition</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>External Disinhibition</td>
<td>2.9 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Overall Disinhibition</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SEM, n =12 NW, n=11 OW/OB, n=23 all subjects. Abbreviations: BMI, body mass index; NW, normal weight; OW, overweight; OB, obese. DEBQ, Dutch Eating Behavior Questionnaire; *Significantly different from NW by independent sample t-test (P<0.05). $^1$Body composition was determined from the sum of skinfold measurements at four points [164].
6.2. Food and Water Intake

6.2.1. Food Intake (kcal)

Preload treatment (P<0.003) but not group (P<0.07) affected energy consumption at the test meal (Table 6.2) and there was no treatment x group interaction (P<0.75). NW girls significantly reduced FI only after 1% chocolate milk treatment compared to water. Because there was no significant effect of group, FI data for NW and OW/OB girls was pooled (n=23) and a one-factor analysis performed. In the pooled sample preload treatment was a factor affecting FI (P<0.002) with 1% chocolate milk (P<0.001) and cola (P<0.02) reducing FI compared to the water control.

In NW girls alone (n=12), preload treatment was a factor affecting FI (P<0.02). FI after 1% chocolate milk was significantly lower than after the water control (P<0.01). FI after cola and fruit drink did not differ from the water control. In OW/OB girls (n=11) none of the caloric beverages resulted in a statistically significant decrease in FI compared to the water control (P<0.19) (Table 6.3).

6.2.1. Cumulative Food Intake (Food + Preload)

Preload treatment (P<0.25) nor group (P<0.06) were factors affecting cumulative (meal plus preload) FI and there was no significant group x treatment interaction (P<0.75). When NW and OW/OB subjects were pooled, preload treatment did not affect FI (P<0.24).
6.2.2. Water Intake

Water intake at the pizza lunch was not affected by the preload treatment (P<0.63) but was affected by group (P<0.01) with higher water intake in OW/OB girls compared to NW girls. There was no treatment x group (P<0.76) interaction (Table 6.2).

6.2.5. Caloric Compensation

Preload treatment (P<0.82) nor group (P<0.50) were factors affecting caloric compensation. Compensation scores were 76, 80, and 85% after the fruit drink, cola, and 1% chocolate milk respectively in NW subjects. Compensation scores were 45%, 73.8% and 59.0% for fruit drink, cola, and 1% chocolate milk respectively in OW/OB girls (Table 6.2). In the pooled sample data treatment did not affect FI (P<0.82).

6.3. Sweetness of Preload Treatments

Preload treatment (P<0.02) but not group (P<0.22) was a factor affecting subjective ratings of sweetness and there was no group x treatment interaction (P<0.79). Subjective sweetness was greater for fruit drink than cola (P<0.02).

6.4. Pleasantness of Preload Treatments

Preload treatment (P<0.001) but not group (P<0.66) was a factor affecting subjective ratings of pleasantness. Subjective pleasantness was greater for the fruit drink treatment in the NW girls (P<0.03) and for 1% chocolate milk treatment in both NW (P<0.003) and OW/OB (P<0.04) subjects compared to the water control. In the pooled sample preload treatment was a factor affecting subjective ratings of pleasantness (P<0.001) with 1% chocolate milk rated significantly more pleasant than cola (P<0.002) and both 1% chocolate milk (P<0.001) and fruit drink (P<0.001) were both significantly higher than the water control (Table 6.3).
6.5. Pleasantness of Test meal

Pleasantness of test meal was not affected by preload treatment (P<0.42) nor group (P<0.08) and there was no group x treatment interaction (P<0.18) (Table 6.2). In the pooled sample pleasantness of test meal was not affected by preload treatment (P<0.45) (Table 6.3).
Table 6.2. Effect of preload treatments on food intake 60 min later in NW and OW/OB girls

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th></th>
<th></th>
<th>OW/OB</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Cola</td>
<td>Fruit Drink</td>
<td>Choc Milk</td>
<td>Water</td>
<td>Cola</td>
<td>Fruit Drink</td>
<td>Choc Milk</td>
</tr>
<tr>
<td>FI¹, (kcal)</td>
<td>935±64</td>
<td>809±65</td>
<td>817±64</td>
<td>746±64†</td>
<td>1066±70</td>
<td>964±92</td>
<td>1010±82</td>
<td>954±68</td>
</tr>
<tr>
<td>Total FP (food+preload) kcal</td>
<td>935±64</td>
<td>967±65</td>
<td>971±64</td>
<td>970±64</td>
<td>1066±70</td>
<td>1122±88</td>
<td>1164±78</td>
<td>1178±65*</td>
</tr>
<tr>
<td>Water Intake, g</td>
<td>140±41</td>
<td>161±39</td>
<td>132±40</td>
<td>145±31</td>
<td>259±47</td>
<td>248±62</td>
<td>230±49</td>
<td>281±42</td>
</tr>
<tr>
<td>CC³, %</td>
<td>-</td>
<td>80±43</td>
<td>76±34</td>
<td>86±16</td>
<td>-</td>
<td>74±29</td>
<td>45±27</td>
<td>59±21</td>
</tr>
<tr>
<td>Preload Sweetness, mm</td>
<td>-</td>
<td>55±10£</td>
<td>74±9§</td>
<td>58±9</td>
<td>-</td>
<td>57±8£</td>
<td>82±4§</td>
<td>71±5</td>
</tr>
<tr>
<td>Preload Pleasantness, mm</td>
<td>34±7</td>
<td>46±9</td>
<td>71±9²</td>
<td>79±8¹</td>
<td>42±8</td>
<td>56±10</td>
<td>66±11</td>
<td>79±7³</td>
</tr>
<tr>
<td>Test Meal Pleasantness, mm</td>
<td>94±3</td>
<td>91±3</td>
<td>92±3</td>
<td>89±4</td>
<td>85±4</td>
<td>82±6</td>
<td>79±4</td>
<td>88±4</td>
</tr>
</tbody>
</table>

Treatment effects were analyzed using the PROC MIXED procedure with preload treatment and group as main factors. Data are means ± SEM; n=12 normal weight and n=11 overweight/obese. Abbreviations: CC, caloric compensation; NW, normal weight; OW, overweight; OB, obese. Means in a row with different letters differ at P<0.05 (Tukey’s test). *Significantly different from NW by unpaired t-test (P<0.05). †P<0.05 versus NW and OW/OB Water; ‡P<0.05 versus NW Water;§ Different than £ within group ¹Pizza intake at test meal. ²Cumulative energy intakes from pizza meal and the preload treatment. ³CC (%) = [Control intake (kcal) – Treatment intake (kcal)]/ kcal in treatment preload] x 100.
Table 6.3. Effect of preload treatments on food intake 60 min later in All Subjects

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Cola</th>
<th>Fruit Drink</th>
<th>Choc Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FI, kcal</strong>¹</td>
<td>997.5±48</td>
<td>883.1±57†</td>
<td>909.6±54</td>
<td>845.2±51†</td>
</tr>
<tr>
<td><strong>Total FI</strong>²</td>
<td>997.5±48</td>
<td>1040.9±57</td>
<td>1063.6±54</td>
<td>1069.2±51</td>
</tr>
<tr>
<td><strong>Water Intake, g</strong></td>
<td>196.8±33</td>
<td>202.3±36</td>
<td>178.9±32</td>
<td>209.6±29</td>
</tr>
<tr>
<td><strong>CC³, %</strong></td>
<td>-</td>
<td>76.8±26</td>
<td>61.4±22</td>
<td>72.3±13</td>
</tr>
<tr>
<td><strong>Sweetness, mm</strong></td>
<td>15.2±5</td>
<td>55.9±6</td>
<td>78.1±5§</td>
<td>63.9±5</td>
</tr>
<tr>
<td><strong>Pleasantness (preload), mm</strong></td>
<td>38±5‡</td>
<td>51±7‡</td>
<td>68±7‡</td>
<td>79±5‡</td>
</tr>
</tbody>
</table>

Pleasantness (test meal), mm: 90±3 86±3 86±3 88±2

Treatment effects were analyzed using the PROC MIXED procedure with preload treatment and group as main factors. Data are means ± SEM; n=23. Abbreviations: CC, caloric compensation; NW, normal weight; OW, overweight; OB, obese. Means in a row with different letters differ at P<0.05 (Tukey’s test). *Significantly different from NW by unpaired t-test (P<0.05). †P<0.05 versus Water; ‡P<0.05 versus Chocolate Milk §P<0.05 versus Cola

¹Pizza intake at test meal. ²Cumulative energy intakes from pizza meal and the preload treatment. ³CC (%) = [Control intake (kcal) – Treatment intake (kcal)/ kcal in treatment preload] x 100.
6.6. Subjective Average Appetite Scores

6.6.1. Average Appetite

Absolute average appetite scores (Figure 6.1a) were affected by preload treatment (P<0.002) and time (P<0.0001), but not group (P<0.85), and there was no group x treatment interaction (P<0.40). Average appetite scores for cola were significantly greater than fruit drink (P<0.016) and water (P<0.015).

Change from baseline average appetite scores (Figure 6.2a) were not affected by preload treatment (P<0.68) nor group (P<0.09) with no group x treatment interaction (P<0.06), but there was an increase over time after all treatments (P<0.0001). Average appetite increased over time for all preloads.

Average appetite scores reported as the change from baseline corrected for the energy content of preload treatment (Figure 6.3a) was not affected by preload treatment (P<0.31), but group (P<0.03) and time were again factors (P<0.0001) with OW/OB girls reported average appetite scores compared to NW girls regardless of preload composition.

6.6.2. Desire to Eat

Absolute desire to eat (Figure 6.1b) was affected by preload treatment (P<0.006) and time (P<0.0001) but not group (P<0.18), and there was no group x treatment interaction (P<0.57). Absolute desire was significantly lower for cola than fruit drink (P<0.05).

Change from baseline desire to eat scores (Figure 6.2b) were also affected by treatment (P<0.002), and time (P<0.0001) but there was no group (P<0.78) or group x treatment (P<0.11) interaction. Subjects desire change from baseline was significantly lower for chocolate milk than fruit drink (P<0.001).

Change from baseline desire scores corrected for the energy content of the preload (Figure 6.3b) was affected by treatment (P<0.0001), and time (P<0.0008) but not group (P<0.6) nor was there a group x treatment interaction.
interaction (P<0.28). Desire scores after chocolate milk were significantly lower than of cola (P<0.03) and fruit drink (0.0001), but fruit drink and cola did not differ significantly from each other (P<0.06).

6.6.3. Hunger

Absolute Hunger (Figure 6.1c) was not affected by preload treatment (P<0.09) or group (0.77) and there was no significant group x treatment interaction (P<0.36) but time was a factor (P<0.0002).

Change from baseline scores (Figure 6.2c) were not affected by preload treatment (P<0.17) but group (P<0.02), and time (P<0.02) were factors, and there was a significant group x treatment interaction (P<0.02). Change from baseline hunger scores were significantly higher for OW/OB girls than NW girls (P<0.006) after fruit drink.

Hunger change from baseline scores corrected for the energy content of the preload (Figure 6.3c) showed no treatment effect (P<0.27) but a weight (P<0.02) was a factor. There was no effect of time (P<0.17) and no weight x treatment interaction (P<0.06).

6.6.4. Fullness

Absolute fullness scores (Figure 6.1d) were not affected by preload treatment (P<0.08) or group (P<0.99) and there was no group x treatment interaction (P<0.77), but time was a factor (P<0.0001).

Fullness change from baseline scores were also not affected by treatment (P<0.07) or group (P<0.69), and there was no group x treatment interaction (P<0.44) but time was a factor (P<0.0001) (Figure 6.2d).

Fullness change from baseline corrected for the energy content of the preload treatment (Figure 6.3d) was not affected by treatment (P<0.09) nor group (P<0.48) and there was no significant group x treatment interaction (P<0.70). Time was a factor (P<0.0002) with fullness scores decreasing over time.
6.6.5. Prospective Food Consumption

Preload treatment (P<0.01), and time (P<0.0001) but not group (P<0.71) were factors affecting absolute prospective food consumption scores (Figure 6.1e) and there was no group x treatment interaction (P<0.66). PFC after the fruit drink was highest compared to the other preload treatments, which was significantly higher when compared to cola (P<0.02), and PFC scores increased over time.

Change from baseline PFC scores (Figure 6.2e) were also affected by preload treatment (P<0.0067) and time (P<0.0001), but not group (P<0.34) and there was no group x treatment interaction (P<0.09). Change from baseline PFC scores were significantly higher for the fruit drink preload than for cola (P<0.03) and chocolate milk (P<0.008) preload treatments.

PFC change from baseline corrected for the energy content of the preload (Figure 6.3e) also showed an effect of preload treatment (P<0.0004) and time (P<0.0001), but not group (P<0.22) and there was no group x treatment interaction (P<0.28). 1% chocolate milk had significantly lower scores than fruit drink (P<0.0005), while fruit drink had significantly higher scores again than cola (P<0.005).

6.6.6. Thirst

Thirst scores were not affected by preload treatment (P<0.15), but group (P<0.001) and time (P<0.0001) were factors affecting thirst scores and there was no significant group x treatment interaction (P<0.69). OW/OB subjects reported higher thirst scores compared to NW girls, and thirst scores for both groups decreased between 0 and 15 minutes. Neither preload treatment (P<0.30) nor group (P<0.25) were factors affecting change from baseline thirst scores, and there was no group x treatment interaction (P<0.92).

6.6.7. Physical Comfort

Preload treatment (P<0.0034), group (P<0.02), and time (P<0.018) were factors affecting absolute physical comfort scores. Physical comfort was significantly lower after cola compared with fruit drink (P<0.018) and 1% chocolate milk (P<0.0067). NW girls had higher PC scores than OW/OB girls. Neither
preload treatment (P<0.16) nor group (P<0.13) significantly affected PC scores but there was a group x
treatment interaction (P<0.009) and time (P<0.03) was a factor. NW girls had significantly lower physical
comfort scores after the 1% chocolate milk compared to OW/OB girls.
Figure 6.1. Absolute appetite scores after caloric treatments to 60 min

Appetite ratings for (a) average appetite, (b) desire-to-eat, (c) hunger, (d) fullness and (e) PFC at 0, 15, 30, 45, and 60 minutes after consumption of the water control, cola, fruit drink, and 1% chocolate milk treatments. Values are means, NW; n=12, OW/OB; n=11. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time (P<0.001).
Appetite ratings for (a) average appetite, (b) DTE, (c) hunger, (d) fullness and (e) PFC at 0, 15, 30, 45, and 60 minutes after consumption of the water control, cola, fruit drink, and 1% chocolate milk treatments. Values are means, NW; n=12, OW/OB; n=11. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time (P<0.001).
Figure 6.3. Change from baseline per kilocalorie preload treatment appetite scores after caloric treatments to 60 min

Appetite ratings for (a) average appetite, (b) DTE, (c) hunger, (d) fullness and (e) PFC at 0, 15, 30, 45, and 60 minutes after consumption of the water control, coke, juice, and chocolate milk treatments. Values are means, NW; n=12, OW/OB; n=11. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time (P<0.001).
6.7. Correlations with Food Intake

6.7.1. Subjective Appetite

6.7.1.1. Average Appetite

Average appetite scores positively correlated with FI at baseline (\(r=0.65, P<0.02\)) and 15 minutes (\(r=0.62, P<0.03\)) after the water preload in NW girls, and at 60 minutes (\(r=0.62, P<0.03\)) after the chocolate milk. Other AA scores did not correlate with FI after any of the preloads in NW or OW/OB girls at any of the measurement intervals following the test beverages.

6.7.1.2. Desire to Eat

Desire to eat scores were positively correlated with FI in NW girls after the water treatment at 0 minutes (\(r=0.59, P<0.05\)) and 30 min (\(r=0.62, P<0.03\)) but not correlated to FI at any other time point for any of the treatments in neither NW nor OW/OB girls.

6.7.1.3. Hunger

Hunger scores were positively correlated with FI in NW girls after the water treatment at 0 (\(r=0.67, P<0.02\)), 15 (\(r=0.67, P<0.02\)), and 30 (\(r=0.63, P<0.03\)) min after the water control, but was not correlated to FI at any other time point for any of the treatments in neither NW nor OW/OB girls.

6.7.1.4. Fullness

Fullness scores were negatively correlated with FI at baseline (\(r=-0.62, P<0.03\)) after the water treatment and at 60 minutes (\(r=-0.66, P<0.02\)) after the chocolate milk treatment in NW girls. OW/OB girls FI was positively correlated with fullness scores at baseline (\(r=0.6, P<0.05\))
after the water treatment. No other fullness scores were significantly correlated with FI for either group at any time point for any preload treatment.

6.7.1.5. Prospective Food Consumption

Prospective food consumption scores were positively correlated with FI at 15 minutes ($r=0.6$, $P<0.03$) after the water treatment, and at 60 minutes ($r=0.58$, $P<0.05$) after the chocolate milk treatment in NW girls. PFC scores were not correlated to FI at any other time point for any of the treatments in neither NW nor OW/OB girls.

6.7.2. Body Composition

Body weight (BW), fat mass (FM) and fat-free mass (FFM) did not correlate with FI or caloric compensation after any of the preload treatments for either NW or OW/OB girls (Table 6.4).
<table>
<thead>
<tr>
<th></th>
<th>NW FI Fruit drink</th>
<th>NW CC Fruit drink</th>
<th>NW FI Choc Milk</th>
<th>NW CC Choc Milk</th>
<th>OW/OB FI Fruit drink</th>
<th>OW/OB CC Fruit drink</th>
<th>OW/OB FI Choc Milk</th>
<th>OW/OB CC Choc Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI Water</td>
<td>0.45</td>
<td>0.56</td>
<td>0.24</td>
<td>0.32</td>
<td>0.11</td>
<td>-0.03</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>FI Cola</td>
<td>0.49</td>
<td>0.56</td>
<td>-0.04</td>
<td>0.35</td>
<td>0.06</td>
<td>-0.14</td>
<td>0.26</td>
<td>0.18</td>
</tr>
<tr>
<td>CC Cola</td>
<td>-0.03</td>
<td>-0.1</td>
<td>0.06</td>
<td>0.18</td>
<td>0.06</td>
<td>0.18</td>
<td>0.06</td>
<td>-0.06</td>
</tr>
<tr>
<td>NW FI Fruit drink</td>
<td>0.24</td>
<td>0.56</td>
<td>-0.26</td>
<td>0.21</td>
<td>-0.68*</td>
<td>0.04</td>
<td>0.26</td>
<td>-0.04</td>
</tr>
<tr>
<td>NW CC Fruit drink</td>
<td>0.28</td>
<td>-0.14</td>
<td>0.04</td>
<td>0.26</td>
<td>-0.11</td>
<td>0.16</td>
<td>0.31</td>
<td>-0.31</td>
</tr>
<tr>
<td>NW FI Choc Milk</td>
<td>0.32</td>
<td>0.35</td>
<td>-0.03</td>
<td>0.49</td>
<td>-0.52</td>
<td>0.04</td>
<td>0.26</td>
<td>-0.18</td>
</tr>
<tr>
<td>NW CC Choc Milk</td>
<td>0.12</td>
<td>0.18</td>
<td>0.47</td>
<td>-0.13</td>
<td>0.11</td>
<td>0.23</td>
<td>0.23</td>
<td>0.40</td>
</tr>
<tr>
<td>OW/OB FI Fruit drink</td>
<td>0.11</td>
<td>-0.03</td>
<td>0.24</td>
<td>-0.08</td>
<td>0.08</td>
<td>0.16</td>
<td>0.15</td>
<td>-0.31</td>
</tr>
<tr>
<td>OW/OB CC Fruit drink</td>
<td>-0.03</td>
<td>0.16</td>
<td>0.15</td>
<td>0.31</td>
<td>-0.31</td>
<td>0.06</td>
<td>0.26</td>
<td>-0.18</td>
</tr>
<tr>
<td>OW/OB FI Choc Milk</td>
<td>0.06</td>
<td>-0.11</td>
<td>0.26</td>
<td>-0.18</td>
<td>0.18</td>
<td>0.23</td>
<td>0.23</td>
<td>0.40</td>
</tr>
<tr>
<td>OW/OB CC Choc Milk</td>
<td>0.04</td>
<td>0.23</td>
<td>0.23</td>
<td>0.40</td>
<td>-0.40</td>
<td>0.23</td>
<td>0.23</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients; n=12 NW, n=11 OW/OB. Abbreviations: BW (kg), body weight; CC, caloric compensation; BMI, body mass index; FFM (kg), fat-free mass; FI (kcal), food intake; FM (kg), fat mass; NW, normal weight; OW/OB, overweight/obese. * significant association at P< 0.05. FM and FFM estimated from the sum of skinfold measurements at four points [164].
6.7.3. Disinhibition, restrained, and emotional eating

Average DEBQ scores did not differ significantly between NW and OW/OB girls (P<0.24) including restrained eating (P<0.89), emotional eating (P<0.28), and disinhibition (P<0.06) scores. Average DEBQ, restraint, overall emotional, diffuse emotional, and specific emotional, nor external or overall disinhibition scores were not significantly correlated to FI or CC for either group, nor when subjects were pooled.
6.7.4 Treatment (kcal) per kg body weight

Treatment (kcal) per kg body weight was inversely associated with FI after the cola treatment in NW girls (P<0.03). No other significant associations between FI and kcal of treatment per KG BW were observed in either group.
Table 6.5. Associations between Treatment Kilocalories Per Kilogram Body Weight and FI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kcal in preload/KG BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td></td>
</tr>
<tr>
<td>FI Cola</td>
<td>-0.63*</td>
</tr>
<tr>
<td>FI Fruit Drink</td>
<td>-0.40</td>
</tr>
<tr>
<td>FI Chocolate Milk</td>
<td>-0.47</td>
</tr>
<tr>
<td>OW/OB</td>
<td></td>
</tr>
<tr>
<td>FI Cola</td>
<td>0.02</td>
</tr>
<tr>
<td>FI Fruit Drink</td>
<td>-0.14</td>
</tr>
<tr>
<td>FI Chocolate Milk</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients; NW; n=12, OW/OB; n=11. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P<0.05
Chapter 7. Discussion
7.1. Discussion

The results of this study support the hypothesis that short-term FI is affected by macronutrient composition and body weight status in girls 9-14 years old. Food intake in NW girls was reduced only after 1% chocolate milk, but not by the other sugars containing beverages and not decreased after any beverage in OW/OB girls suggesting that body weight status and composition affected the response to the caloric beverages in 9-14 year old girls.

A suppression of FI by 1% chocolate milk was expected in both NW and OW/OB girls for the following reasons. First, a stronger effect of protein on FI suppression has been observed compared to carbohydrate or fat [193]. Of the 13 grams of protein found in the 1% chocolate milk preload, 20% or approximately 3 grams of this was whey protein which has been shown to reduce FI and enhance satiety in both adults [70] and children [7]. For example, preloads of whey protein suppressed FI to a greater extent than sucrose, casein, egg albumin, or soy protein [71, 74] in adults. The effect of whey protein on FI may be related to its amino acid composition [194, 195]. Whey is digested rapidly resulting in increases in plasma amino acid concentrations which are sustained for up to two hours [92] which is associated with satiety [196]. Ingestion of whey protein has been also been observed to result in the release of gut hormones involved in satiety, specifically to increase plasma concentrations of CCK [92] and GLP-1 [101], and decrease ghrelin concentrations when compared to glucose in adult males [101, 197].

Casein comprises the remaining 80% of the 13 g of protein found in the 1% chocolate milk, and has also been shown to contribute to satiety in humans [89]. Most research suggests that caseins role in satiety is observed in longer rather than shorter term [92], however a recent study demonstrated caseins effect on satiety and short-term FI [198] where a 20 g casein beverage increased satiety and reduced FI to a greater extent than whey, albumin, and
maltodextrin treatments at a test meal 30 min later in men [198]. The difference between whey, a “fast” protein and casein, a “slow” protein, as described by Boirie et al [89] rests in the differences in digestion and absorption. Casein, unlike whey, is coagulated in the stomach, resulting in a slower rate of gastric emptying resulting in a lower amino acid concentration compared to whey [92]. The resulting differences in CCK and GLP-1 concentrations, observed to be higher after whey compared to casein preload treatments, may contribute to the suppression of short-term FI more frequently observed after whey [92].

Energy content of the beverages in this study are lower than many other reports of short-term FI on the effect of macronutrient source [71, 92, 199] and may have diminished the effect of commercial beverages on FI in this study. The higher energy content of the 1% chocolate milk may have contributed to the greater reduction of FI in the NW girls. At 224 calories per 350 ml the 1% chocolate milk provided 29% and 31% greater energy than the cola and fruit drink treatments respectively. 1% chocolate milk treatment in the NW girls provided the greatest amount of energy per kg of BW (5.68 kcal/kg BW) and more than any beverage in OW/OB girls. The failure of OW/OB girls to reduce FI after the 1% chocolate milk and the other beverages may have been related to the relatively lower energy dose to BW.

In the pooled sample a reduction in FI after 1% chocolate milk and cola but not fruit drink was observed despite the similar energy content between cola and fruit drink, suggesting that composition was the stronger determinant of FI. A higher glucose to fructose ratio has been shown to be a factor on the effect on FI in adults, but the results from this study suggest that fructose fraction of the sugars beverages may be the primary component leading to the suppression of FI. In the present study the cola treatment lead to a significant decrease in FI, and had a lower glucose to fructose ratio of 0.7 compared to the fruit drink of 1.1.
In addition, cola had a significantly lower pH at 2.96 than both fruit drink (pH = 2.46) and 1% chocolate milk (pH=6.77). The acidity of the cola causes the hydrolyzation of bonds between glucose and fructose in the beverage, leaving free monosaccharides available to be more rapidly absorbed which may then contributed to reductions in FI [69]. Other individual properties of the treatments may have also contributed to their effects on FI including level sweetness [57], carbonation [200] and caffeine levels of the cola, and the presence of calcium and cocoa powder in the 1% chocolate milk. Although the independent effect of the foregoing factors could not be determined from the current study design.

Body composition was measured in the study because in previous research FM has been shown to affect CC after caloric preloads in boys [7, 39]. In 3-5 y old girls FM was inversely associated with CC after carbohydrate beverages [39]. However results from the current study differ from this previous research, as no consistent association between FM, BMI, or FFM and FI was found after any of the preload treatments in either group. There was a trend for OW/OB girls to show lower caloric compensation than NW girls, and although this difference was not significant in the present study, it suggests that hormonal mechanisms of FI may be compromised in the OW/OB girls compared to the NW girls. Power analysis suggest that a sample size of ~175 girls would be needed to detect a difference in CC between NW and OW after the 1% chocolate milk with a power of 0.8. When NW and OW/OB were pooled a sample size of ~200 subjects was required to detect a difference between CC between treatments.

In adult women CC has been shown to be lower than men [95]. In one short-term FI study, compensation comparing orange juice, semi-skimmed milk, and fruit drink ranged from 57% for orange juice, 85% for semi-skimmed milk, and 7% for sugar-sweetened fruit drink in female subjects, while in males CC of ~100% were reported for all treatments [95]. Similarly
when a yogurt preload was compared to a water control in women, CC scores were consistently lower in women versus men. [148]. The difference in the regulation of FI between genders may be explained by differences in the concentration of satiety hormones such as ghrelin [124] and GLP-1 [69]. Sex differences in the release of appetite stimulating hormones ghrelin and PYY have been confirmed through the study of opposite sex twins with higher total plasma ghrelin and lower PYY secretion in women compared to men [201], suggesting that women would suppress FI less than men.

Restrained eating has been a factor hypothesized to have an effect on FI [202]. High levels of cognitive restraint are hypothesized to interfere with the ability to accurately regulate FI [142]. The Dutch Eating Behavior Questionnaire was used to assess emotional and external disinhibition, and restrained eating. High scores on DEBQ have been associated with increased risk of being overweight [142]. Women have been observed to score higher than males on restrained and emotional eating [142]. In the current study however NW and OW/OB girls DEBQ scores did not differ. Furthermore no association was observed between DEBQ scores and FI or CC.

Subjective appetite was measured after each of the preload treatments at baseline and 15 min intervals to lunch at 60 min. Neither treatment nor group were strong factors affecting FI in these girls which is consistent with previous research demonstrating no difference among subjective appetite scores after sugars containing beverages in adult women [69]. This is in contrast to our other published studies in boys [7, 25] as well as young girls [160, 185] where we consistently showed a relationship between subjective appetite and FI. Weaker associations between subjective appetite and FI has been reported in adult women [86].
Both desire to eat and PFC were significantly lower after 1% chocolate milk in NW girls when corrected for the energy content of the preload. This suggests that on a per kcal basis 1% chocolate milk is a potent regulator of both appetite and FI in NW girls. Additionally, no significant association was observed between the energy content of the treatment per kg BW and FI after 1% chocolate milk in both NW an OW/OB girls, supporting the hypothesis that macronutrient composition is the strongest determinant of FI in girls.

Total cumulative FI, including energy from the treatment as well as intake from the pizza meal, after all caloric treatments did not differ significantly from the water control. Therefore, the current recommendation to replace sugars containing beverages with water or non-caloric alternatives is unlikely to have an impact on promoting healthier body weights in girls. The significant reduction of FI after 1% chocolate milk in the NW girls, as well as its’ observed effects on subjective appetite, demonstrate that 1% chocolate milk is a strong regulator of FI, and should be tested in longitudinal studies to assess the effect on weight maintenance or weight loss.
7.2. Methods and Limitations

Preload treatments were provided at a fixed volume of 350 ml for all NW and OW/OB subjects. This amount provided 158, 154, and 224 kcal of cola, fruit drink, and 1% chocolate milk, respectively. This fixed volume however may not have allowed for accurate comparisons across groups as NW subjects received a higher dose per kg body weight than OW/OB girls. In NW boys 10-13 years of age who were given caloric beverages on a BW basis, both whey and glucose resulted in decreased FI compared to a control at a pizza lunch 60 min later, with a greater suppression of FI after the whey compared to glucose [7]. The fixed volume dose was chosen for this study however, to provide a comparison among subjects as would be observed in a real world setting, based on the common portion sizes found in regular size soft drinks.

Neither average appetite nor any individual subjective appetite scores were consistently associated with FI. The weak relationship between FI and appetite scores may be interpreted by some to suggest that children do not understand how to correctly fill out the VAS to reflect their appetite. However, stronger associations between average appetite, desire-to-eat and hunger with FI in post-pubertal, but not peripubertal girls, suggests subjective appetite may be a learned association as older girls more accurately make this connection [185]. The girls in this study were younger and primarily peripubertal compared to those in the previous study. Desire to eat, hunger, and PFC decreased, and fullness significantly increased after the test meal suggesting that the girls understood the questions, which has also been observed previously with children of the same age [7, 25].
7.3. Conclusion

In conclusion, most sugars containing beverages suppressed FI, but the response was affected by macronutrient composition, energy content of the beverages, and body weight status.

7.4. Future Directions

7.4.1. Sex Differences

Different areas of the brain respond to hunger and satiety cues in females [203] which may produce different eating responses. In addition to observed sex differences in the release of hormones such as ghrelin [124], levels of cognitive restraint and concentrations of reproductive hormones differ between males and females, which may be involved in satiety. Future research is needed to identify further the differences between male and female mechanisms of satiety.

7.4.2. Gastrointestinal Hormone Differences between OW/OB and NW

The differences observed in the response of NW and OW/OB subjects in this as well as previous research studies [3, 7] warrants further investigation. As the differences in the NW and OW/OB girls could not be attributed to variations in levels of restraint or body fatness, future research is suggested to determine the differences in the secretion of satiety related hormones in response to macronutrient composition. OW/OB individuals have previously been observed to have lower levels of ghrelin [140] and lower fasting concentrations of PYY [119]. Future research investigating short-term compensation to energy preload treatments should include measurement of hormones known to effect regulation of FI such as insulin, leptin, Cholecystokinin, PYY, and GLP-1 [105]. As FM was not correlated with FI in the current research, future research is needed in girls to identify the role of gastrointestinal hormones in NW and OW/OB girls to establish the cause of observed differences in FI.
8.0. References


Chapter 9. Appendices
Effect of Commercially Available Beverages on Short-Term Food Intake in Children

Recruitment Letter for Parents

Dear Parent

Mount Saint Vincent University is leading a team of researchers investigating the physiological and environmental determinants of energy intake regulation on the health of children and young adolescents. In our current work we are conducting studies aimed at understanding the controls of food intake in children, with the ultimate goal of finding ways to address the problems of overeating and obesity that are becoming a concern among those people involved in improving the long term health of Canadians.

We are asking the parents of boys and girls 9 to 14 years old to allow their children to take part in a research study. Their participation is quite straightforward: on four separate weekend mornings, following a 12 hour fast, your child will consume a standard breakfast at home, and then consume a sweet beverage followed by a pizza lunch 60 minutes later in the Department of Applied Human Nutrition, Mount Saint Vincent University. The study will take place on four weekend mornings at the Evaristus Building (Room 365), Department of Applied Human Nutrition.

There are criteria for participation that you need to be aware of, the child must:
- be between 9 and 14 years of age, and
- be healthy, and have been born at term, and
- not be taking medications.
- not have allergies to milk, wheat or nuts.

If you would like your son or daughter to participate, or to get further information beyond that provided in this letter, please contact Dr. Nick Bellissimo, Principal Investigator, Ms. Lorianne Bennett (Project Coordinators) at (902) 457-6378 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,

Dr. Nick Bellissimo, Department of Applied Human Nutrition, Mount Saint Vincent University.
Ms. Lorianne Bennett, Department of Applied Human Nutrition, Mount Saint Vincent University.
Appendix 9.2. Study Information Sheet and Parent’s Consent Form

Department of Applied Human Nutrition

Effect of Commercially Available Beverages on Short-Term Food Intake in Children

Study Information Sheet and Parent’s Consent Form

Investigators:

Dr. Nick Bellissimo, PhD, BEd
Principal Investigator
Phone: (902) 457-6295
Email: nick.bellissimo@utoronto.ca

Ms. Lorianne Bennett
Project Coordinator
Phone (902) 457-6378
Email: lorianne.bennett@msvu.ca

Invitation:

Mount Saint Vincent University is leading a team of researchers investigating the physiological and environmental determinants of energy intake regulation on the health of children and young adolescents. In our current work we are conducting studies aimed at understanding the controls of food intake in children, with the ultimate goal of finding ways to address the problems of overeating and obesity that are becoming a concern among those people involved in improving the long term health of Canadians. We are asking the parents of 9-14 year old boys and girls to allow their children to take part in a research study.

Purpose of Research:

The purpose of this study is to determine the effects of beverages on food intake regulation in normal weight and overweight/obese 9-14 year-old boys and girls. This experiment is being conducted through the Department of Applied Human Nutrition at Mount Saint Vincent University by Dr. Nick Bellissimo, Ms. Lorianne Bennett. Your child will be required to attend four experimental sessions conducted over a 4-week period, for a total of 5 visits (4 food intake measurement sessions + 1 information/screening visit) to the Mount Saint Vincent
University campus. Each visit will last approximately 90 minutes.

Procedure:

Appetite Assessment:

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

During the information/screening session, the researcher will explain the full details of the study. Parents that give consent to have their child participate will sign a consent form. The parent will receive copies of consent forms and of the study information sheet. If the child wishes to participate and signs a children’s assent form, their weight, height, and body fat by skinfold caliper at 4-points (biceps, triceps, supra-iliial, and subscapular), will be measured.

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

The children who participate in this study will be requested to go to the Evaristus Building (Rm. 365), Department of Applied Human Nutrition, Mount Saint Vincent University, for four individual weekend morning sessions over a four week period.

On each of the four test days, the children will have a standardized breakfast of cereal, milk and orange juice at home, either at 8:00 am, 9:00 am or 10:00 am (the time will be consistent for each child). The children will arrive at the Evaristus Building, either at 10:00 am, 11:00 am or 12:00 pm (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).

Each child will receive 350 mL of either bottled spring water, 1% chocolate milk, fruit punch or soft drink (e.g. Coca Cola). Each child will receive all drinks, one on each day in no set order.

McCain pizza and spring water (purchased at Sobey’s or Atlantic Superstore) will be served 60 minutes after the children have consumed their beverage. Children will be told that they may eat as little or as much as they like. The amount of food eaten by each child will be measured.

The children will also be requested 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”), thirst (“Not thirsty at all” to “As thirsty as I ever felt”) sweetness of the drinks (“Not sweet at all” to “Extremely sweet”), pleasantness of the preload and pizza (“Not at all Pleasant” to “Very Pleasant”, and physical comfort (“Not
They will complete these scales during the information/screening session, in order to become familiar with the test instruments. The children will be fully supervised during the study sessions. They will be engaged in age appropriate entertainment (as distraction) such as reading, playing puzzles or card games before lunch.

Eating Behaviour Questionnaire:

If you consent to your child’s participation in this experiment, he/she will also be asked to fill out a short questionnaire about their eating habits during the information/screening visit or after one of the food intake sessions. A trained examiner will help your child fill out the questionnaire. The answers will be strictly confidential and will only serve to assist in the analysis of the data collected. Your child may skip any questions of the questionnaire that make them feel uncomfortable.

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 completion of the study and then securely destroyed.

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:

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Dr. Nick Bellissimo or Ms. Lorianne Bennett at (902) 457-6378.

Dissemination of findings:

A summary of results will be made available to you to pick up, or if requested will be sent by mail or e-mail, 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 $10 Empire Theatre gift certificate. I will also receive $5 to cover transportation costs following each study session. 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.

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

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 9.3. Children’s Assent Form

Effect of Commercially Available Beverages on Short-Term Food Intake in Children

Children’s Assent Form

Purpose of Research:
The purpose of this study is to determine the effects of beverages on appetite in children. My weight, height, and body fat will be measured during the information/screening visit. I will also be required to drink a different beverage (within 5 minutes) each week, and complete special scales to show if I am hungry or full during each session. I will fill-out a short questionnaire about my eating behaviours, and know that I am allowed to skip any questions that may make me feel uncomfortable. I will also be provided with a pizza lunch at the end of each study session (that I will eat in the Department of Applied Human Nutrition, Mount Saint Vincent University). All the experimental sessions will be on weekends, 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.

Also, if at any time I decide to stop participating, that will be O.K. I understand that information related to me will be kept confidential. I know that I will receive a $10 Empire Theatre gift certificate after completion of each study session, as a “thank you” for my participation.

“I was present when ______________________________read this form and gave his/her
__Signature

Name of the person who obtained assent:
____________________________

Date: _________________ (dd/mm/yy)
Appendix 9.4. Telephone Screening Questionnaire

Telephone Screening Questionnaire

MOUNT SAINT VINCENT UNIVERSITY

Food intake control in children. 

Name: ____________________

Age: _________ Years 

DOB (d/m/y) _________ 

Term baby? yes/no

Height: _________ cm. 

Weight: _________ kg. 

Normal birth weight? yes/no

Has your child gained or lost weight recently? yes/no (circle correct answer)

Does your child usually have breakfast? yes/no

Does your child like (foods that will be used in experiments 1, 2, 3 & 4)

- Fat-free/ chocolate milk yes/no
- soft drinks (e.g. coke) yes/no
- Juice (fruit punch, orange) yes/no
- Pizza yes/no

Is your child following a special diet? yes/no

Does your child have food allergies or sensitivities?

Milk, nuts, wheat yes/no

Health problems? yes/no

If yes, which problem? ____________________________
Medication/s? yes/no
If yes, which medication/s? __________________

Education: Grade: _________ Special class? yes/no

Skipped or repeated grade? yes/no Learning difficulties/problems? yes/no

Behavioral or emotional problems yes/no
If yes, which problem? __________________________
__________________________________________

Include in study? yes/no
If not, why? __________________________

Appointment date: _________ (d/m/y)

Investigator: ________________ Date: _________ (d/m/y)
Appendix 9.5. Recruitment Poster

Mount Saint Vincent University

ATTN: PARENTS OF CHILDREN AGED 9-14 YEARS

We are conducting a nutrition research study in children.

REQUIREMENTS: healthy, 9-14 year old boys and girls

INVOLVES: 1 screening + 4 weekend sessions (breakfast & lunch included)

As a reward for taking part, at each session the child will receive a theatre movie pass. Parents will receive $5 travel reimbursement after each session.

For more information, please contact Kelly Poirier or Lorraine Bennett at 902-157-6378.
Appendix 9.6. Study Day Questionnaires

VAS – Motivation to eat

VAS – Pleasantness (preload beverage)

VAS – Pleasantness (pizza lunch)

VAS – Perceived Sweetness

VAS – Physical Comfort

Feeding Session Cover Sheet

Dutch Eating Behaviour Questionnaire
Appendix 9.6a VAS motivation to eat

Visual Analogue Scale

Motivation to Eat

DATE: ________________________

ID: ________________________

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

1. How strong is your desire to eat?

Very ________ Very

WEAK ______________________ STRONG

2. How hungry do you feel?

NOT ________ As hungry

Hungry ______________________ as I have

at all ever felt

3. How full do you feel?

NOT ________ VERY

Full ______________________ Full

at all

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

NOTHING ________ A LARGE

at all ______________________ amount

5. How thirsty do you feel?

NOT ________ As thirsty

thirsty ______________________ as I have

at all ever felt
Appendix 9.6b VAS pleasantness of preload

Visual Analogue Scale
Pleasantness of Preload

DATE: ________________________
ID: ________________________

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

How pleasant have you found the preload?

NOT ___________________________ Very pleasant
at all
pleasant
Appendix 9.6c VAS pleasantness of test meal

Time =

Visual Analogue Scale

Pleasantness of Test Meal

DATE: _______________________

ID: _______________________

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 food?

NOT _______________________________________________________ Very
at all
pleasant
Appendix 9.6d VAS Sweetness

Time =

Visual Analogue Scale
Sweetness

Subject ID: ________________________

Date: ________________________

Please rate the level of sweetness by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

How sweet have you found the beverage?

NOT _______________________________ Extremely sweet
sweet at all
Appendix 9.6e VAS Physical comfort

Visual Analogue Scale

Physical Comfort

DATE: ________________________

ID: ________________________

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

1. How well do you feel?

<table>
<thead>
<tr>
<th>NOT</th>
<th>VERY</th>
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<tr>
<td>well at all</td>
<td>Well</td>
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</table>

| _____________________________ | _____________________________ |
| _____________________________ | _____________________________ |
Appendix 9.7 Feeding Session 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:
Appendix 9.8 - Dutch Eating Behaviour Questionnaire

Please read each question and circle the appropriate response.

1. If you have put on weight, do you eat less than you usually do?
   Never   Seldom   Sometimes   Often   Very often

2. Do you try to eat less at meal times than you would like to eat?
   Never   Seldom   Sometimes   Often   Very often

3. How often do you refuse food or drink offered because you are concerned about your weight?
   Never   Seldom   Sometimes   Often   Very often

4. Do you watch exactly what you eat?
   Never   Seldom   Sometimes   Often   Very often

5. Do you deliberately eat foods that are slimming?
   Never   Seldom   Sometimes   Often   Very often

6. When you have eaten too much, do you eat less than usual the following day?
   Never   Seldom   Sometimes   Often   Very often

7. Do you deliberately eat less in order not to become heavier?
   Never   Seldom   Sometimes   Often   Very often

8. How often do you try not to eat between meals because you are watching your weight?
   Never   Seldom   Sometimes   Often   Very often

9. How often in the evenings do you try not to eat because you are watching your weight?
   Never   Seldom   Sometimes   Often   Very often

10. When you eat, do you take into account what you weigh?
    Never   Seldom   Sometimes   Often   Very often

11. Do you have the desire to eat when you are irritated?
    Never   Seldom   Sometimes   Often   Very often

12. Do you have the desire to eat when you have nothing to do?
<p>| | | | | |</p>
<table>
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<tbody>
<tr>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
</tr>
</tbody>
</table>

13. Do you have the desire to eat when you are depressed or discouraged?

14. Do you have a desire to eat when you are feeling lonely?

15. Do you have a desire to eat when somebody lets you down?

16. Do you have a desire to eat when you are angry?

17. Do you have a desire to eat when you are expecting something unpleasant to happen?

18. Do you get the desire to eat when you are anxious, worried or tense?

19. Do you have a desire to eat when things are going against you or when things have gone wrong?

20. Do you have a desire to eat when you are frightened?

21. Do you have the desire to eat when you are disappointed?

22. Do you have a desire to eat when you are bored or restless?

23. Do you have a desire to eat when you are emotionally upset?

24. If food tastes good to you, do you eat more than usual?

25. If food smells and looks good to you, do you eat more than usual?
26. If you see or smell something delicious, do you have the desire to eat it?

Never  Seldom  Sometimes  Often  Very often

27. If you have something delicious to eat, do you eat it straight away?

Never  Seldom  Sometimes  Often  Very often

28. If you walk past the baker, do you have the desire to buy something delicious?

Never  Seldom  Sometimes  Often  Very often

29. If you walk past a snackbar or a cafe, do you have the desire to buy something delicious?

Never  Seldom  Sometimes  Often  Very often

30. If you see others eating, do you also have the desire to eat?

Never  Seldom  Sometimes  Often  Very often

31. Can you resist eating delicious foods?

Never  Seldom  Sometimes  Often  Very often

32. Do you eat more than usual when you see others eating?

Never  Seldom  Sometimes  Often  Very often

33. When your mother or father are preparing a meal, are you inclined to eat something?

Never  Seldom  Sometimes  Often  Very often