Mount Saint Vincent University
Department of Applied Human Nutrition

Effect of Sugars-Sweetened Commercial Beverages on Subjective Appetite and Short-Term Food Intake Regulation in Normal Weight and Overweight/Obese 9-14 Year Old Boys

by

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Abstract

This study was conducted to test the hypothesis that 1% chocolate milk will increase meal-time satiation and decrease short-term food intake (FI) to a greater extent than other isovolumetric sugars-sweetened beverages in normal weight (NW) and overweight/obese (OW/OB) boys, although the effect will be diminished in OW/OB boys. The primary objective was to determine the effect of isovolumetric (350 ml) preloads of fruit drink, cola and 1% chocolate milk on short-term FI and subjective appetite when compared to a water control in 9-14 year old NW and OW/OB boys. On four separate mornings and in random order, boys consumed a calorie-free water control, fruit drink (154 kcal), cola (158 kcal) or 1% chocolate milk (224 kcal) beverage 2 hours after a standardized breakfast. Boys significantly reduced FI after cola (NW: 894 ± 54; OW/OB: 986 ± 75) and 1% chocolate milk (NW: 844 ± 52; OW/OB: 912 ± 63) compared to a water control (NW: 1046 ± 51; OW/OB: 1050 ± 49). Caloric compensation (CC) scores were not significantly different between groups for the fruit drink (NW: 44% vs. OW/OB: 16%, p=0.56), cola (NW: 96% vs. OW/OB: 40%, p=0.27) or 1% chocolate milk (NW: 90% vs. OW/OB: 61%, p=0.24) treatments. When corrected for the energy content of the treatment, fullness was higher after cola (p=0.02), and prospective food consumption (PFC) lower after 1% chocolate milk (p=0.009) compared to the fruit drink. PFC and DTE were the strongest and weakest predictors of FI, respectively. BW was positively associated with FI and inversely associated with CC in OW/OB, but not NW boys (P<0.05). In OW/OB, the treatment dose of cola (kcal/kg BW) was inversely associated with FI (P<0.05). In conclusion, cola and 1% chocolate milk suppressed FI in boys, however, the effect on FI was dependent on macronutrient composition, treatment dose and body composition.

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List of Abbreviations

A	
AA	Average appetite
ANOVA	Analysis of variance
B	
BG	Blood glucose
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BW	Body weight
C	
CC	Caloric compensation
CCK	Cholescytokinin
CNS	Central nervous system
D	
DEBQ	Dutch Eating Behaviour Questionnaire
DTE	Desire to eat
DXA	Dual energy x-ray absorptiometry
F	
FFM	Fat-free mass
FI	Food intake
FM	Fat mass
G	
GI	Glycemic index
GLP-1	Glucagon-like peptide-1
GMP	Glycomacropeptide
H	
HFCS	High-fructose corn syrup
N	
NW	Normal weight

O

OB	Obese
OW	Overweight

P

PFC	Prospective food consumption
PYY	Peptide tyrosine tyrosine

S

SEM	Standard error of mean
SSB	Sugar sweetened beverage

V

VAS	Visual analogue scale

W

WPI	Whey protein isolate

Chapter 1. Introduction

1.1. General Introduction

Obesity is one of the major threats to public health in Canada [1]. Over the past three decades, the prevalence of obesity among Canadians has increased, with the rise in children of particular concern [1]. Prevention of childhood obesity is a high priority because obese children have an increased risk of developing chronic diseases later in life including cardiovascular disease, hypertension and diabetes [2, 3]. Obese children are also more likely to become obese as adults [2, 3] and have increased rates of morbidity and mortality in adulthood [4, 5]. The development of these chronic diseases increases the financial burden on the healthcare system.

Environmental factors have been the overwhelming focus of research investigating the causative factors of obesity in children [6] although less is known about the physiological mechanisms of food intake regulation. It is unknown whether obesity develops in susceptible individuals because their physiological mechanisms of food intake control are compromised first, or whether these mechanisms are present but become overridden by environmental factors and then become compromised [7]. Few studies have investigated food intake regulation in children by measuring food intake at test meals following the consumption of energy containing beverages.

The increased consumption of sugars-sweetened beverages has been hypothesized to contribute to increasing rates of childhood obesity as the sugars contained in these beverages are thought to bypass food intake regulatory mechanisms leading to an increase in consumption [8]. Although the availability of soft drinks in Canada increased between 1980 and 1998, rates have declined over the past decade [9]. Further, 2-5 year olds have demonstrated a precise ability to compensate for the calories contained in sugars-sweetened beverages opposing the hypothesis that sugars contained in these beverages bypass food intake regulatory mechanisms [10]. Less is known about the ability of older children to compensate for calories contained in sugars-sweetened drinks.

Over the same time period that overweight and obesity rates have increased, the consumption of fluid milk has also declined. The replacement of milk with sugars-sweetened beverages in the diet may affect overall energy intake as the high protein content of milk has been observed to enhance satiety [11] more than sugars-sweetened beverages alone which contain carbohydrate only. Previous research conducted in 9-14 year old boys demonstrated the suppressive effect of whey protein in normal weight but not overweight boys suggesting that

differences may exists between the physiological mechanisms controlling FI in normal weight and obese individuals [12].

Further research is needed to determine the effect of macronutrients on FI when consumed in their commercially available form as this will help determine the specific role of macronutrients on short-term food intake regulation in children. This study will be the first to compare the effect of isovolumetric (350 ml) preloads of fruit drink, cola and 1% chocolate milk on subjective appetite and short-term FI when compared to a water control in 9-14 year old NW and OW/OB boys.

Chapter 2. Literature Review

2.1. Introduction

The following literature review is comprised of five sections and will provide relevant background for the research study conducted. The first section provides an introduction of the diagnosis, prevalence, consequences and causes of childhood overweight and obesity. The second section describes the effect of macronutrient composition on short-term FI regulation. The third section describes the physiological control of FI including a brief description of the mechanisms involved in carbohydrate, protein and fat induced suppression of FI. The fourth section is a description of the research literature as it relates to gastrointestinal hormone regulation of FI in children and the final section provides a general overview of the test methods utilized to measure short-term FI and body composition in children and adolescents.

2.2. Childhood Obesity

2.2.1. Diagnosis of Obesity in Children and Adolescents

According to the Centers for Disease Control (CDC) and Prevention, body mass index (BMI) is an index for height and weight which serves as a reliable indicator of fat mass (FM) [13]. Although BMI does not measure body fat directly, it has been correlated with direct measures of body fat including underwater weight and dual energy x-ray absorptiometry (DXA). BMI is also used to screen for weight categories that may increase individual's risk of developing to health problems [13]. There are several methods available for diagnosing obesity including bioelectric impedance (BIA), DXA and underwater weighing. However, these methods are expensive and are not readily accessible; therefore BMI is commonly used to assess individual's BW status. In adults, "underweight" is classified as a BMI less than 18.5, "normal weight" is classified as a BMI between 18.5-24.9, "overweight" is classified as a BMI between 25-29.9 and "obese" is defined as a BMI greater than 30 [14]. The United States Centers for Disease Control (CDC) and Prevention BMI growth charts are recommended to screen children and adolescents for obesity [13]. In Canada, the CDC growth charts are used to determine body weight (BW) status in 2-18 year olds [13]. These charts compare children's BMI to gender specific BMI-for-age values to obtain a percentile ranking. "Normal weight" is defined as a BMI between the 5^{th} percentile and 85^{th} percentile for height, weight and gender. "Overweight" is defined as a BMI between the 85^{th} and the 95^{th} percentile and "obese" is defined as a BMI above the 95^{th} percentile [13].

2.2.2. Prevalence of Overweight and Obesity in Children and Adolescents

Over the past three decades, overweight and obesity rates among children have more than doubled in Canada [15]. According to the 2004 Canadian Community Healthy Survey, the combined overweight and obesity rate among Canadian children aged 2-17 was 26% [16]. During the past 25 years, national rates of overweight and obesity among adolescents have increased from 14% in 1978-79 to 29% in 2004 and obesity rates have tripled from 3% to 9% [16].

2.2.3. Consequences of Obesity in Children and Adolescents

Childhood obesity is linked to a multitude of physical, psychosocial and economic consequences [17] including an increased risk of developing type II diabetes, hypertension, hyperlipidemia [1, 18], coronary heart disease [19, 20], stroke, gallbladder disease, osteoarthritis and sleep apnea [20]. Obese children are also at an increased risk of becoming OB adults [2, 3] and have increased rates of morbidity in childhood and adulthood [4, 5]. Further, OB children are more likely to have poor self-esteem and an undesirable body image [21] which is linked to feelings of sadness, loneliness and nervousness [22]. They are also more prone to engage in risk behaviors including smoking and alcohol consumption [22]. In 2006, the subsequent health costs associated with being OW and OB were estimated to account for $6.0 billion dollars in total direct healthcare costs contributing to 4.1% of the total healthcare expenditures in Canada [23]. Trends suggest that this amount could increase substantially to account for over $8 billion in the near future [23].

2.2.4. Causes of Overweight and Obesity

Obesity is a complex disease resulting from genetic [24] and environmental factors [25, 26]. Bouchard and Tremblay et al. [24] investigated the role of genetics in the development of obesity by overfeeding 12 pairs of adult monozygotic twins by 1000 kcal per day, 6 days a week for a total of 84 days during a 100-day period. Each set of twin demonstrated a significant response to overfeeding for BW, percentage of fat, FM and estimated subcutaneous fat ($P<0.05$) with approximately three times more variance among than within pairs. The intra-pair similarities regarding the ability to adapt to long-term overfeeding suggests that genetic factors are involved in the physiological regulation of BW [24]. While evidence continues to emerge regarding the role of genes in regulating body fat, environmental factors continue to be the main

focus of research investigating the causative factors of obesity [6].

In general, obesity is a result of a positive energy balance when energy intake exceeds energy expenditure [27]. The presence of a positive energy imbalance has been linked to a variety of environmental factors including the increased availability of sugars in the food supply [28], increased portion sizes of inexpensive appetizing foods [29], increased consumption of take-out foods [30], decreased levels of physical activity [31] and increased television viewing [32]. Details regarding how these factors contribute to obesity will be discussed below.

Between 1971 and 1997, the availability of sugars in the United States increased by 30% [33] which has been hypothesized to play a role in positive energy balance [34]. However, between 1994 and 2010, sugars and syrups consumption decreased in Canada while obesity rates have continued to rise [35]. The hypothesis that sugars are the only factor contributing to childhood obesity is unlikely for several reasons. First, in addition to the increased availability of sugars in the food supply, there has been a proportionate increase per capita in the availability of poultry (85%), cooking oils (47%), dairy products (424%), vegetables 73% and energy (15%) [33]. However, because food availability data is reported as the amount of food available per capita for consumption, it does not consider food wastage or spoilage and therefore may overestimate the amount of food consumed by the population.

The majority of research surrounding the association between sugars consumption and obesity has been epidemiological and observational and therefore cause and effect cannot be determined. Furthermore, higher sugars intakes have actually been associated with lower BW [36]. Data collected between 1994-2010 within the National Population Health Survey and the Canadian Community Health Survey support a negative association between sugars and syrups consumption and obesity rates in Canada. Furthermore, data from the 2004 Canadian Community Health Survey found that total energy intake rather than the macronutrient composition of the diet significantly increased the risk of developing obesity in adults [35].

Over the past 35 years, the portion sizes of many foods have increased significantly [37]. Many of these foods are available in larger portion sizes and they are also inexpensive and appetizing [29] contributing to their increased consumption. Not only are increasing portion sizes a concern but also the types of foods that are being consumed. Between 1977 and 1998, the portion sizes for foods consumed both inside and outside the home increased for salty snacks, desserts, soft drinks, and hamburgers [38]. In one study, children consumed more macaroni and

cheese when provided with a large compared to a small portion [39]. These results are consistent with another study in which children consumed 25% more of an entree when they were given a large portion on a plate compared to when they were able to choose their portion [40].

Since the 1970's, the prevalence of take-out food consumption has increased [30] and approximately 75% of adolescents consume fast food one or more times per week [41]. Several studies have found a direct association between fast food consumption and BW [42, 43] while others have not [41, 44]. A recent study found that OB children ate more on days when they consumed a fast food meal (2703 kcal/day) compared to days when they did not (2295 kcal/day) [7] while another suggested that the frequency of fast food consumption was associated with a less nutritious diet among adolescents [41].

Sedentary activities including television viewing, video gaming and computer usage have become more common among Canadian children and may increase the risk of becoming OW or OB [45] by decreasing the amount of time spent being physically active. On average, Canadian children spend 2.25 hours on weekdays and 3.75 hours on weekends watching television or playing video games [46]. In 2004, 36% of children 6-11 year of age engaged in more than two hours of screen time each day. These children were twice as likely as to be OW and OB compared to those who participated in screen time activities for an hour or less daily (35% vs. 18%) and twice as likely to be OB (11% vs. 5%) [16]. Television viewing is associated with increased energy intake as well as a reduced ability to control FI among young boys [47]. A study conducted in 9-14 year old boys investigated the effect of television viewing on FI at a test meal 30 min after consuming a caloric (glucose) or non-caloric preload. Boys consumed 228 kcal more after the television viewing condition suggesting that television viewing during meal time contributes to increased energy intake by delaying satiation and reducing satiety signals from foods consumed before the activity [47]. The combination of sedentary activities and an increased consumption of energy-dense foods helps to promote weight gain [48].

2.2.5. Sugars-Containing Beverages and Childhood Obesity

Over the past two decades, the consumption of sugars-sweetened beverages (SSB) in the United States increased significantly [49]. Several cross-sectional and observational studies link soft drink consumption with greater energy intake and BW as well as inadequate nutrition [50, 51]. The 1994-96 Continuing Survey of Food Intake by Individuals found that soft drinks provide the greatest source of sugar in the diet of adolescents accounting for 36.2 g/day in girls

and 57.7 g/day in boys [52]. According to Statistics Canada, Canadians consumed 110 g of sugars/day accounting for 21.4% of their total daily energy intake in 2004 [35]. Absolute sugar consumption was the highest among boys aged 9-18 years and the sugars contained in beverages accounted for 44% of their average daily sugar intake. Of the total sugars consumed from beverages; 14% originated from milk, 14% from soft drinks, 9% from fruit juice and 7% from fruit drinks [35].

Various cross-sectional studies in children [30, 53, 54] have found associations between the consumption of SSB, weight gain [55, 56], obesity [57] and percent body fat [30]. Berkey et al. (2004) [58] investigated the relationship between changes in BMI and intakes of SSB among a large sample of 9-14 year olds over two, one year periods. For each additional daily serving of SSB, BW increased by 100 g over one year [58] suggesting that SSB contribute to a modest increase in BW. Another study in 4-16 year olds found that OW children consumed significantly more SSB than their NW counterparts although they also consumed more meat and grain products, potato chips and sugar [30]. While studies have shown a positive relationship between SSB and FI [55], others have failed to show associations between SSB and BW status [59, 60].

2.3. Effect of Macronutrient Composition on Food Intake Regulation

Food consumption initiates physiological responses which control FI regulation and stimulate satiety [61]. Evidence suggests that the macronutrient composition [62] rather than the physical composition of a food has a greater ability to regulate appetite and FI [63]. A review article by Stubbs et al. (2000), found that protein, carbohydrate and fat exert hierarchical effects on satiety in the following sequence; protein > carbohydrate > fat [64]. However, there is limited research regarding whether this macronutrient hierarchy persists in children [65].

Infants and children have the ability to adjust FI in response to differences in the macronutrient as well as the energy content of foods, when they are allowed to self-regulate their FI without the presence of external cues [66]. Young children also respond to the energy density of foods and eat in response to hunger and satiety cues. At a young age, external factors begin to affect children's hunger and satiety cues including the time of day, availability of palatable food, emotional state and current dieting behaviours [66]. Young children adjusted their FI to compensate for 110 kcal differences in puddings provided 25-30 min before a meal [67]. In another study, 3-5 year olds compensated for calories contained in high and low-energy preloads at a meal 25 min later; energy intake was 237 kcal and 128 kcal after the high and low-

energy treatments respectively [67]. Similarly, the energy density of preloads had no effect on subsequent FI in adults; energy intake was 390 kcal following both high-energy and low-energy preloads [67]. In another study, 9-10 year olds did not reduce FI in response to sucrose (210 kcal) or aspartame preloads provided 90 min before a meal [68]. The findings of these studies suggest that individuals differ in their ability to adjust FI depending on their age, the energy and macronutrient contents of the preloads and the time interval between consumption of the preload and the test meal.

2.3.1. Carbohydrates and Short-Term Food Intake Regulation

Carbohydrates are the main source of energy in the human diet representing 45-65% of the daily total energy intake. Sugars suppress short-term FI [69] although their effect is dependent on the source and dose of the sugar preload as well as the timing relative to the measurement of FI. Carbohydrate containing preloads as well as meals composed of glucose, sucrose or fructose decrease short-term FI by an amount approximately equal to their energy contents [10, 70, 71]. Several experimental studies indicate that sucrose containing beverages suppress FI in preschool children [72] and adults [73-75] although the effect is dependent on the source of the carbohydrate contained in the preload [69].

2.3.1.1. Sugars-Sweetened Beverages and Short-Term Food Intake

Research is lacking regarding the effect of commercially sweetened beverages on short-term FI regulation in children. Soft drinks have been described as thirst-quenching liquids [76] that bypass satiety mechanisms controlling FI [8]. Fructose and glucose, the two monosaccharides that form sucrose, are not absorbed until sucrose is hydrolyzed by intestinal brush-border enzymes [77]. However, when contained in a soft drink, a proportion of sucrose is hydrolyzed into glucose and fructose by the acidic pH of the beverage before it is consumed [77]. This increases the absorption rate of the sugars perhaps reducing subsequent FI. Several studies in adults have failed to find significant differences in FI following the consumption of caloric beverages. Adults had no differences in FI 50 min after an equicaloric preload containing sucrose or high-fructose corn syrup (HFCS) and FI after an isoenergetic isovolumetric milk preload was not different than after a sucrose or a HFCS preload [77]. Similarly, young men had no significant difference in FI 30 min after consuming 500 ml of cola or chocolate milk [11]. Among women, FI did not differ after consuming 360 g of water, diet cola, regular cola, orange

juice or 1% chocolate milk. Evidence suggests that liquid preloads providing less than 200 kcal do not suppress FI because the quantity is less than the threshold required for detection by physiological hunger mechanisms [70].

2.3.1.2. Sucrose

Sucrose is a disaccharide composed of equal parts of fructose and glucose linked by alpha-1-4-glycosidic bonds [55]. In children [78] and adults [70, 75, 79], sucrose has been shown to suppress short-term FI and appetite. Young males (18-35 y) substantially reduced FI 80 min after a 300 ml sucrose preload compared to an equivolumetric, sweet, energy-free control [80]. Men also reduced FI 60 min after 50 g [81] or 75 g [75] sucrose preloads. Conversely, males provided with sucrose preloads differing in energy content (83 kcal vs. 166 kcal) did not adjust FI at test meals 30 or 60 min later [82]. Evidence suggests that sucrose preloads composed of 50 g or more of sucrose provided 20-60 min before a meal will reduce FI [70]. For example, a 75 g sucrose preload reduced FI to a greater extent than 25 and 50 g sucrose preloads in young males [75].

It is hypothesized that SSB contribute to excess FI through by-passing FI regulatory mechanisms [34, 83]. However, several short-term FI studies have found that individuals have a remarkable ability to reduce FI at test meals 30-60 min after sugar-containing preloads [73]. Children (2-5 y) reduced energy intake by the same amount of calories contained in a 90 kcal sucrose preload at a test meal provided 30 min after consuming the preload [10] indicating that liquid sucrose treatments have the ability to suppress short-term FI [10].

Although not as precise, older children also reduce FI based on the caloric content of sucrose preloads. The ingestion of a cherry-flavoured drink containing 45 or 90 g of sucrose reduced FI 30 min later in 9-10 year olds [65]. Alternatively, 9-10 year olds did not adjust FI at a test meal provided 90 min after 300 ml sucrose or aspartame-sweetened beverages differing by 210 kcal [68]. Sucrose provided in liquid preloads has been shown to suppress subjective appetite as well as reduce FI in children although there is limited research regarding the satiating ability of other sugars including glucose or fructose in children.

2.3.1.3. Glucose

In comparison to sucrose, glucose preloads have been observed to reduce short-term FI in children [12] although evidence is conflicting. NW boys (9-14 y) reduced FI after a 50 g glucose

preload compared to a sweetened water control [12]. Caloric compensation (CC) scores following a 50 g glucose preload were 106% and 81% for NW and OW boys respectively [12] which is greater than the response which has been previously observed in NW adult men [28]. This suggests that young children have a greater ability to adjust FI in response to calories consumed as carbohydrate compared to adults [84].

In adults, healthy males demonstrated greater CC after a glucose 80, fructose 20 (G80:F20) preload compared to a G20:F80 preload (92% vs. 45%) [80]. Similarly, young males reduced their FI following a 75 g glucose preload compared to a sucralose control. In contrast, adults consumed significantly more calories after a 50 g glucose compared to a 50 g fructose preload, an aspartame preload or a water control ($P<0.05$) [85].

2.3.1.4. Fructose

Fructose has been shown to increase satiety and decrease FI to a greater extent than glucose [86]. A 50 g fructose preload reduced FI more than a glucose preload at test meals provided 38 min [85] and 145 min later [86] in adults. In contrast, young men consumed significantly less calories after a G80:F20 preload compared to a G20:F80 preload; 1046 kcal vs. 1207 kcal [80]. The ability of fructose to enhance satiety is attributed to several factors. First, fructose is absorbed more slowly than glucose in the gastrointestinal tract [87] resulting in a longer contact time with gastrointestinal receptors which initiate satiety signals [88]. In addition, fructose is not absorbed completely in the gastrointestinal tract which creates a hyperosmolar environment in the large intestine. This may result in malaise or diarrhea and subsequently reduce FI [89]. However, if fructose is consumed with other carbohydrates, it is absorbed more quickly [90]. For example, when fructose is consumed with small amounts of glucose, it is rapidly transported to the intestine [87].

2.3.2. Protein and Food Intake Regulation

Protein is well known to be the most satiating macronutrient [91-96]. In a recent review paper by Eisenstein et al. (2002), 8 out of 10 preload experiments demonstrated lower FI after a high compared to a low-protein preload [97]. The protein source, quantity and composition as well as the time until the next meal are important factors to consider when investigating the effect of protein on short-term FI [98]. Adults reduced FI and reported greater ratings of subjective satiety 90 min after a 48 g whey protein preload compared to a 48 g casein preload

[99]. Although protein is well known to have the strongest ability to suppress FI relative to carbohydrate and fat, a recent study found that protein source was also a determinant of FI [98]. Young men fed 45-50 g preloads composed of egg-albumen, whey, soy or sucrose 60 min before a meal reduced FI the most after the whey and soy protein preloads relative to a water control [98]. The egg albumen preload lead to a significantly greater cumulative FI compared to the control [98]. Conversely, protein source was not a factor affecting FI when provided in a meal [100]. When six dietary proteins (egg albumen, casein, gelatin, soy protein, pea protein and wheat gluten) were included in a dinner meal, FI did not differ 8 h later at a supper meal [100]. These results may be attributed to the low concentration of the test proteins provided to subjects relative to the total energy content in the lunch meal (22% of energy as protein) as well as the 8 h time period between the dinner and supper meals [98].

Quantitative, and subjective measures of appetite suggest that dietary protein regulates short-term satiety, suppresses FI and delays hunger [98, 101] more than carbohydrate [98] and fat [96, 102] in adults [103]. When 12 females were given equicaloric preloads composed of protein, carbohydrate, fat and alcohol; FI and subjective hunger decreased while satiety increased significantly after the protein preload [104]. Other studies have found contradicting results. Young women reported decreased ratings of hunger and desire to eat (DTE) after a protein juice drink compared to a juice drink containing fat or carbohydrate, although there were no differences in FI at a subsequent dinner meal [105].

In children, there is evidence that the consumption of meals high in protein increases satiety more than meals high in carbohydrate [106]. However, a study conducted in 9-14 year old boys illustrated the suppressive ability of whey protein in NW but not OB boys [12]. CC scores after a 50 g whey protein preload were 91% and 39% in the NW and OB boys respectively [12]. Similarly, in the Iowa breakfast studies, adolescent girls were provided with breakfasts' differing in protein contents (9, 15 or 24 g) for six days. Subjects who consumed the high-protein breakfast (24 g) experienced a 17% reduction in daily energy intake compared to when they consumed the low-protein breakfast (9 g) [107]. Unfortunately, the sample size was small (n=8).

There are several mechanisms that explain the effect of protein on suppression of FI. Protein has the ability to stimulate diet-induced thermogenesis (DIT) [92, 105] which is responsible for several energy-dependent processes which occur during the post-prandial period, including the intestinal absorption of nutrients, the initial steps of metabolism as well as the

storage of absorbed nutrients [108]. Depending on the amount of energy required to complete the initial steps of metabolism, DIT differs for each nutrient. Protein has a thermic effect of 20-30% which is greater than carbohydrate (5-15%) and fat (0-3%) [32] resulting in greater energy expenditure during the post-prandial period. High protein foods have also been shown to increase thirst leading to a possible reduction in overall hunger [109]. There is limited research however regarding the satiating ability of protein in a liquid form.

2.3.2.1. Cow's Milk

Several observational studies have demonstrated an inverse relationship between dietary calcium intake from dairy products and FM in women [110] and children [111, 112]. The precise mechanisms behind calcium's ability remain unknown. Cow's milk contains several components that play an important role in satiety including casein, whey and glycomacropeptide (GMP). Fluid milk is an aqueous medium composed of water (88%), carbohydrate (5%), protein (3.5%) and fat (3.3%). One cup of cow's milk contains approximately 11-12 g of carbohydrate [113]. When consumed with carbohydrate, milk proteins reduce short-term appetite [61, 98, 114] and BG concentrations [115, 116].

Milk is hypothesized to be more satiating than carbohydrate containing beverages including soda and juice because of its high protein content, although studies comparing milk and SSB's exhibit varied results [8]. In adults, FI did not differ at a lunch meal provided 135 min after consuming 590 ml preloads of sparkling water, orange juice, 1% milk and cola despite greater ratings of fullness and reduced ratings of hunger and DTE after the three energy-containing beverages [8]. Similarly, young men (20-40 y) did not reduce FI at a test meal provided 30 min after 500 ml of chocolate milk despite increased ratings of satiety [11].

Research has also investigated the effect of chocolate milk on FI. Cocoa powder contained in chocolate milk has been shown to play a role in insulin secretion [117]. In one study, chocolate milk led to a 45% increase in insulinemia compared to strawberry flavored milk although there were no significant differences in FI [117]. It is hypothesized that insulinogenic compounds in cocoa powder simulate β-cell secretion [118]. High insulin responses are associated with short-term appetite regulation and increased satiety [118]. While protein is more satiating than carbohydrate or fat, studies have yet to provide evidence that 1% milk suppresses short-term FI [119] or that flavored milk increases overall satiety.

2.3.2.2. Whey Protein

Research has shown that dairy products and their components suppress short-term FI and stimulate physiological mechanisms which initiate satiation and satiety [120]. There are two main dietary proteins found in milk; whey and casein. Whey protein accounts for approximately 20% of cow's milk [63] and functions to enhance satiety and suppress FI [98]. Whey protein has a strong effect on FI for several reasons [12]. First, whey protein is rapidly digested [121] leading to a quick sustained increase in plasma amino acids which has been observed to suppress FI [122]. Second, whey protein releases several gut peptides including cholecystokinin (CCK) [123], glucagon-like peptide-1 (GLP-1) [72], and peptide tyrosine tyrosine (PYY) from intestinal tract cells [124] which initiate satiety signals to suppress FI. Whey protein also contains a high content of branched chain amino acids which stimulate insulin release [61, 125]. Insulin in turn increases short-term satiety and decreases FI.

Several studies have found that whey protein (40-60 g) provided in a liquid preload reduces subsequent FI [98, 99]. In adults, a whey protein preload (37.7g/300 ml) reduced FI by 80 kcal more than a carbohydrate preload and 56 kcal more than a control suggesting that liquid whey protein enhances satiety to a greater extent than carbohydrate [91]. Similarly, 5 g and 10 g whey protein preloads as well as a 10 g whey protein hydrolysate preload reduced FI and post-meal BG response in a dose dependent manner among healthy adults [125]. Compared to casein, whey has a greater ability to suppress short-term FI and enhance subjective satiety [99]. Men fed flavored beverages composed of whey protein, soy protein and egg albumen isolates followed by a pizza meal 1-2 h later decreased FI the most after the whey preload compared to a water control [98]. Similarly, adults consumed less at a test meal after a whey preload compared to a casein preload (879 kcal vs. 1084 kcal) [99]. Research has yet to investigate whether casein would have a greater effect on long-term satiety compared to whey protein.

The effect of whey protein on FI has only recently been investigated in children. Bellissimo et al. conducted a study to compare to the effect of glucose and whey-protein on subjective satiety and short-term FI in 9-14 year old boys [12]. Following a 50 g glucose preload, NW and OB boys reduced their FI at a pizza meal similarly; CC scores were 106% and 81% in NW and OB boys respectively. However, OB boys did not reduce FI significantly after the whey protein preload. CC scores after the whey preload were 39% in OB boys compared to 91% in NW boys ($p < 0.05$) [12]. It is unknown why the effect of the whey protein preload on FI was

compromised in the OB boys although it was hypothesized to be related to potential differences in the release and/or the sensitivity to satiety hormones between the groups [12].

2.3.2.3. Casein Protein

Casein, accounts for approximately 80% of the protein content of cow's milk [125, 126]. Casein protein is has been observed to have a weaker effect on satiety compared to whey protein [99]. For example, adults had a significantly lower cumulative energy intake at an ad libitum buffet meal 90 min after a 48 g whey protein preload compared to after an isoenergetic casein preload [99]. Similarly, adults reported to be significantly less hungry after consuming a breakfast composed of whey protein than after a breakfast composed of casein or soy at a concentration of 10% of energy from protein [127]. However, there were no differences in energy intake at a subsequent lunch meal [127].

This is explained by differences in the digestive and absorptive properties of whey and casein protein [121]. Casein is labeled as a 'slow' protein [99] as it coagulates in the stomach from the precipitation of gastric acid [126] and is digested gradually [61]. This increases the time required for gastric emptying resulting in a smaller postprandial rise in plasma amino acids. In comparison, whey is referred to as 'fast protein' [99] as it is digested more rapidly [61] which increases the rate of amino acid absorption [121]. According to Mellinkoff's amniostatic theory [128], a rise in plasma amino acids increases satiety [99] therefore whey protein would be expected to enhance satiety and suppress FI more quickly than casein.

2.3.2.4. Glycomacropeptide

GMP, also referred to as caseinomacropeptide is a peptide which originates from the κ-casein protein which accumulates in the whey fraction during cheese making [129]. GMP functions as a secretagogue of CCK [129] although its ability is dependent on the GMP variant as well as glycosylation of GMP [129]. Caseinomacropeptide, the unglycosylated form of GMP is also a potent stimulator of CCK, a hormone which stimulates satiety [130, 131]. In humans, research investigating the effect of GMP on subsequent FI varies. OW females were significantly less hungry and reduced FI after a whey protein preload enriched with GMP and oleic acid [132]. Conversely, objective and subjective measures of satiety were not different in adults who consumed 0.4 g or 2.0 g of GMP in a 100 ml preload [133]. However, the GMP dose may have been below the threshold required to significantly reduce FI. Similarly, healthy adults had greater

ratings of fullness after consuming 300 ml beverages composed of whey protein isolate with 21% GMP as well as a mixture of whey protein isolate and 21% GMP compared to whey protein isolate extracted from skim milk, although FI did not differ [130]. In humans, little research has been conducted to investigate the effect of GMP on FI, CCK release and CCK-mediated satiety [130]. Further, the ability of whey protein to stimulate the release of CCK and function in CCK-mediated satiety depending on the GMP content of the whey protein remains unknown [129].

2.4. Fat and Short-Term Food Intake

Excessive fat intake is often linked to increasing obesity rates [134]. However, several cross sectional and longitudinal studies have failed to show a consistent relationship between dietary fat intake and FM [135, 136]. Investigating the impact of fat intake on satiety and FI is important because much of the variability in food's energy density results from differences in fat content.

Comparisons have shown that fat is less [137, 138] satiating than carbohydrate [95, 139]. Several studies in young children have found a reduction in short-term FI after consuming fat. In one study, preschool children were provided with four different preloads; fat-free frozen dessert (0 g fat), medium fat ice-cream (12 g fat), high fat ice-cream (18 g fat) and a baseline snack of cheerios and apple juice (0 g fat) which was followed by an ad libitum lunch [140]. Energy intake at the test meal was almost equivalent with 249, 257 and 254 kcal consumed after the fat-free, medium-fat and high-fat preloads respectively [140]. This suggests that children respond to high fat foods by decreasing subsequent FI [140]. In contrast, young men provided with safflower oil preloads containing 0, 100, 200 and 300 kcal suppressed FI only after the 300 kcal preload [75]. This indicates there is a higher caloric threshold for foods containing fat which must be ingested to affect FI. Similarly, women reported to be slightly more hungry and less satiated following a high-fat preload compared to a high-carbohydrate preload despite no significant differences in FI at a subsequent test meal [104]. Previous research regarding milk's role in satiety has focused on its high protein content rather than the fat content.

2.5. Effect of Time to the Next Meal on Food Intake

The findings of preload studies are often dependent on the time interval between administration of the preload treatment and consumption of the test meal [8, 73, 141]. Previous research in adults has utilized time intervals ranging from 20 min to several hours [8]. In

children, researchers have used time intervals of 20-30 [47], 30 [10], 60 [10] and 90 [68] min.

The ability to regulate FI after the consumption of protein and carbohydrate preloads is dependent on the time interval between ingestion of the preload treatment and ingestion of the test meal. Previous studies comparing the effect of carbohydrates and whey protein on short-term FI in adults have utilized time intervals of 60 min or greater [74, 75, 98]. The ability of protein and carbohydrate to suppress FI is similar when assessed at an ad libitum test meal 60 min after consumption of the preloads [142]. Protein however has a greater ability to suppress FI at time intervals >120 min [95, 104, 143]. After longer time intervals (1-5 h), the accuracy [141] and precision [144] of CC decreases irrespective of the preload's macronutrient composition. In one study, NW adults accurately compensated for high-carbohydrate and high-fat yogurt preloads after a 30 min delay. However, when the time interval was increased to 90 and 180 min, CC decreased [141]. Conversely, a 50 g whey protein preload suppressed FI more than a glucose preload 60 but not 30 min later in 9-14 year old NW and OW boys [12]. This study utilized a 60 min time interval between consumption of the treatments and the test meal to allow subjects to differentiate between the effects of carbohydrate and protein on subsequent FI [12].

2.6. Physiological Control of Food Intake

The balance between energy intake and energy expenditure as well as the initiation and termination of meals is managed by a variety of interactions between satiety hormones and the central nervous system (CNS). The hormonal regulation of FI has been investigated in adults although research is limited in children. The human gastrointestinal tract releases short and long-term hormones which play a role in signaling FI and in turn affect overall energy balance. FI is also dependent on macronutrient content [141] and there is evidence to suggest that protein is more satiating than carbohydrate which is more satiating than fat [98].

2.6.1. Obesity and Short-Term Food Intake Regulation

The effect of childhood obesity on the regulation of short-term FI has received little investigation although body fat stores have been associated with the failure to adjust FI in young children [145]. A study among children (3-5 y) found that FM measured by skinfold analysis was inversely related to FI regulation at a subsequent test meal in girls but not boys 20 min after a high-calorie preload [145]. Boys compensated for a significantly greater percentage of energy compared to girls; 55% vs. 35% [145]. Further, CC was inversely related to FM in girls but not

boys [145]. The short-term regulation of FI is has been observed to be more accurate in NW individuals compared to those who are OB which suggests that FM may play a role in diminishing satiety signals leading to increased FI. For example, compared to NW boys, OW/OB boys were unable to suppress FI after a 50 g whey protein preload [12].

Excess amounts of FM are hypothesized to modify the relationship between satiety hormone concentrations and appetite therefore short and long-term satiety hormones may perhaps differ in NW and OB individuals. High-fat meals increase plasma ghrelin [146], a gastric hormone responsible for meal initiation [147]. OB individuals often fail to experience a calorie-dependent suppression in postprandial circulating ghrelin levels [47] which indicates that they are insensitive to the actions of ghrelin. Furthermore, OB individuals are more likely to be insulin resistant which is linked to the inability to accurately control appetite. In one study, hyperinsulinemic men did not experience equivalent decreases in subjective appetite scores 15 min after a glucose preload compared to normoinsulinemic men which suggests that hyperinsulinemia plays a role in the failure of individuals to decrease appetite after consuming glucose [126].

2.6.2. Mechanisms of Carbohydrate Induced Satiety

The consumption of carbohydrates enhances satiety signals [148] which often differ depending on the composition and dose of the carbohydrate [149]. Carbohydrates have been observed to decrease subjective appetite and FI at subsequent test meals [148]. Carbohydrate induced satiety is dependent on the interaction of nutrients with receptors in the gastrointestinal tract which control FI [148]. The ingestion of carbohydrates increases blood glucose (BG) concentrations and stimulates the release of gastrointestinal hormones including GLP-1 and amylin [148]. These hormones regulate a variety of functions including gastric emptying, intestinal transit as well as the perception of gastric and intestinal distension [150]. The ability of carbohydrates to regulate short-term FI is inversely related to the glycemic [74] and insulin [151] response to sugars. Subjective appetite and FI are both inversely related to BG [74, 75], insulin [152], leptin [153] and CCK [154] concentrations.

In 1953, Mayer proposed the glucostatic theory of FI regulation to explain how carbohydrates regulate FI [155]. This theory suggests that low BG concentrations stimulate feeding while high BG levels signal satiety and therefore terminate feeding. Consistent with this theory is the ability of carbohydrates to increase BG concentrations which enhances satiation

[156]. Several studies have found transient declines in BG concentration following meal initiation [90, 157]. Researchers have also investigated the association between BG and FI regulation using the glycemic index (GI). This index is used to describe the rise in BG following a meal [83]. This theory proposes that high GI foods lead to a rapid increase in circulating BG levels and a subsequent rise in insulin response which is followed by a rapid decline in BG levels leading to hunger [10]. In contrast, consumption of a low GI food results in a lower more sustained increase in BG and therefore reduced insulin secretion. Consistent with this theory, Ludwig et al. (1999) [83] found that OB boys consumed 53% more after consuming high-GI instant oatmeal compared to medium-GI steel-cut oatmeal [83]. However, in the short-term (i.e. 1 h), high-GI carbohydrates reduce subsequent appetite and FI while low-GI carbohydrates delay satiety (i.e. 2-3 h) [90]. A review article found no association between BG concentrations before an ad libitum meal and FI suggesting that insulin as opposed to glucose, is involved in the short-term regulation of FI [118]. Conversely, Anderson et al. (2002) found that high-GI preloads (glucose, polycose and sucrose) reduced FI to a greater extent at a test meal 60 min later compared to low-GI preloads (amylose, amylopectin and a fructose-glucose mixture) [74] indicating an inverse relationship between BG concentrations and FI [74].

2.6.3. Mechanisms of Protein Induced Satiety

In the short-term, dietary protein maintains satiety over a longer time interval compared to the other macronutrients [158]. However, similar to carbohydrate [73], the amount of protein in addition to the time until the next meal are important factors which affect the ability of protein to suppress FI [98]. A six month randomized trial found that high-protein diets enhanced satiety more than low-protein diets [159]. However, the ability of high-protein diets to reduce the risk of developing obesity is unknown because many high-protein diets are also high in fat [97].

There are a variety of mechanisms involved in the suppression of FI resulting from protein [98]. First, protein digestion promotes the release of satiety hormones from the gastrointestinal tract [98] including CCK, GLP-1, PYY and ghrelin [61]. There is also evidence that the peptide products created by protein digestion reduce FI by slowing stomach emptying [160] and stimulating gut hormones including CCK [160] and GLP-1. Free amino acids resulting from protein digestion are absorbed by the blood and directly regulate FI by stimulating FI regulatory mechanisms in the CNS [61].

2.6.3.1. Mellinkoff's Amniostatic Theory

Protein's satiating ability also differs depending on the type of protein. For example, casein is labeled as a 'slow' protein [99] because it is digested gradually [61] resulting in a small postprandial rise in plasma amino acids. In comparison, whey is referred to as 'fast protein' [99] because it is digested more rapidly [61] which increases the rate of amino acid absorption in the gastrointestinal tract [121]. According to Mellinkoff's amniostatic theory [128], an increase in plasma amino acids enhances subsequent satiety [99]. This is supported by a study in which adults were fed normal-casein (10% energy from protein) and high-casein (25% energy from protein) breakfasts [27]. Adults experienced a significant increase in subjective satiety 3 h after the high-casein breakfast. However, FI was similar after the normal-casein and high-casein breakfasts. Amino acid concentrations were also measured and the largest increase occurred 3-4 h after breakfast. In support of Mellinkoff's theory, it was hypothesized that the prolonged elevation of plasma amino acid concentrations may have contributed to the increased satiety ratings after ingestion of the high-casein breakfast [27].

2.6.4. Mechanisms of Fat Induced Satiety

Research has yet to investigate the effect of fat on satiety in children. In one study, young men consumed more energy after a safflower oil preload compared to after an equicaloric sucrose preload; 884 kcal vs. 778 kcal [75]. Fat's role in satiety is attributed to its ability to stimulate CCK [88], a satiety hormone released from the mucosal cells in the small intestine which helps mediate fat-induced satiety [161]. Fat also slows gastric emptying which increases nutrient's contact time with gastrointestinal receptors in the stomach [88]. Based on the physiological mechanisms involved in fat-induced satiety as well as its high energy density, fat is hypothesized to contribute to energy imbalance to a greater extent than other macronutrients. Furthermore, signals from fat metabolism and storage are hypothesized to be more appropriate for the long-term regulation of energy balance.

2.7. Hormonal Regulation of Food Intake

There are a variety of gastrointestinal peptides which contribute to satiety including CCK from the duodenum, GLP-1 from the ileum as well as insulin and glucagon from the pancreas [162]. Most recently, PYY has been found to contribute to FI regulation. PYY is secreted from the gut proportionately to the amount of calories consumed [163]. The long-term regulation of

energy homeostasis is regulated by adipose stores and energy intake over a prolonged time period. Insulin and leptin are responsible for regulating long-term FI and maintaining energy balance [162].

2.7.1. Short-Term Gastrointestinal Hormones

There are a variety of anorexigenic and orexigenic hormones responsible for regulating satiety and short-term FI. The ability of macronutrients to suppress FI is dependent on the satiety hormones they release. Carbohydrates ingested orally or administered directly into the stomach or small intestine reduce FI [148]. Compared with a water control, 25, 50 and 75 g sucrose preloads reduced FI at a test meal 60 min later in young men [75]. The ability of carbohydrates to induce satiety is mediated by changes in glucose, GLP-1, amylin and CCK concentrations [148]. These hormones regulate gastric emptying, intestinal transit as well as the perception of gastric distension [150, 164]. Young men decreased FI at a test meal 60 min after consuming high GI preloads (glucose, polycose and sucrose) compared to low GI preloads (amylose, amylopectin and a fructose-glucose mixture) suggesting that FI and subjective appetite are associated with BG response [74].

The mechanisms involved in proteins' ability to suppress short-term FI to a greater extent than carbohydrate and fat [95, 96] are dependent on the production of satiety hormones in the gastrointestinal tract [66]. In NW and OW males, liquid preloads of soy, whey and gluten prolonged postprandial suppression of ghrelin, elevated CCK and GLP-1, and maintained BG levels compared to a glucose preload [66]. In adults, fat stimulates the release of CCK although fat-induced satiety in children has received little exploration [165]. Several short-term gastrointestinal hormones will be discussed in the following section.

2.7.1.1. Cholecystokinin

CCK is a satiety hormone [166] released from endocrine cells in the small intestine [162] in response to meals containing fat. CCK mediates fat-induced satiety by reducing meal size [162]. Rats given CCK before a meal experienced a dose-dependent decrease in meal size [167]. Similar experiments demonstrated the ability of CCK to reduce meal size in humans [168, 169]. Exogenous and endogenous CCK play a role in suppressing FI in many species by stimulating CCK_1 receptors [162]. The administration of specific CCK_1 receptor antagonists before a meal increases meal size in humans which suggests that endogenous CCK stimulates satiety [170].

Long-term peripheral administration of CCK however does not reduce FI or help sustained weight loss [171]. Although CCK is associated with satiation as evidenced by meal termination, the presence of an inverse relationship between CCK and energy intake that almost reached significance (P=0.06) suggests that CCK may also contribute to satiety [72]. In humans, dietary protein is intermediate to fat and carbohydrate in terms of stimulating the release of CCK [172] and whey protein is a stronger stimulator than casein protein [99]. Healthy adults demonstrated a 60% increase in CCK after a 48 g whey preload compared to an equivalent amount of casein [99]. Similarly, CCK secretion was extended after liquid preloads of soy, whey, and gluten compared to after a glucose preload [173]. This indicates that CCK plays an important role in protein-induced satiety.

2.7.1.2. Glucagon-like Peptide-1

GLP-1 is a satiety hormone, which reduces appetite and FI [174]. In humans, intravenous administration of GLP-1 induces satiation [175] by inhibiting gastric emptying [150]. The secretion of GLP-1 is influenced by macronutrient composition. A 75 g glucose preload was observed to stimulate GLP-1 secretion to a greater extent than an equivolumetric fructose preload [176]. In men, postprandial GLP-1 secretion was prolonged after 50 g whey, soy or gluten protein preloads compared to after a 50 g glucose preload suggesting that GLP-1 also mediates protein-induced satiety [66].

Both carbohydrate [175] and fat are effective stimulators of GLP-1 [61] which is released when glucose comes in contact with L-cells in the small intestine [177]. GLP-1 may also play a role in milk protein induced satiety [61]. A study in rats demonstrated that Exendin-4, a GLP-1 receptor antagonist interacted with milk proteins (casein and whey) to suppress subsequent FI [178]. Whey protein is a strong stimulator of GLP-1 and its secretagogue effect is hypothesized to be enhanced by the presence of other macronutrients [61]. A high-protein breakfast (58% of total energy) enriched with whey protein isolate induced higher GLP-1 concentrations over a 3 h period compared to a high carbohydrate breakfast (19% of total energy from protein) composed predominantly of casein protein [179]. This evidence provides another plausible mechanism for the ability of whey protein to increase satiety and suppress FI [98].

2.7.1.3. Peptide Tyrosine Tyrosine

PYY is a gastrointestinal hormone that was first discovered by Tatemoto [180]. Its name

reflects the presence of an amino acid terminal tyrosine and a carboxyl terminal tyrosine amide [181]. PYY is released postprandially from L-cells in the gastrointestinal tract [63] depending on the amount of energy contained in a meal [182] as well as the rate of gastric emptying. PYY functions as a short-term satiety signal [182] by terminating feeding [163]. This is achieved by slowing gastric emptying and delaying gallbladder and pancreatic secretions [183]

The two main forms of endogenous PYY are PYY_{1-36} and PYY_{3-36} [63]. In humans, the amounts of these forms of PYY differ depending on the feeding status [163]. In a fasted state, the concentration of PYY_{1-36} is greater than that of PYY_{3-36}. However, following a meal, PYY_{3-36} becomes the major circulating form [163]. Postprandially, plasma levels of PYY increase within 15 min, reach a maximum at 90 min and remain elevated for up to 6 h [184]. Fat is the strongest stimulator of PYY while the levels of PYY reflect the meal size and composition [163].

Although research investigating the role of PYY is limited, this hormone is observed to enhance short-term satiety and reduce FI by inhibiting neuropeptide Y activity [163]. Twice daily administration of PYY_{3-36} for 8 days in rats decreased cumulative FI and reduced BW gain compared to a saline treatment [163] supporting the role of postprandial PYY in FI regulation. Similarly, adults who received a 90-min infusion of PYY_{3-36} (0.8 pmol/kg/min) followed by an ad libitum lunch 2 h after termination of the infusion exhibited a 36% reduction in FI after the PYY_{3-36} infusion compared to a saline solution [163]. During the post-infusion period, subjects who received the PYY_{3-36} infusion experienced a 33% reduction in total 24-h FI [163] suggesting that PYY_{3-36} is released in proportion to the amount of calories ingested.

2.7.1.4. Ghrelin

Ghrelin is an orexigenic gut hormone that stimulates appetite [185] and regulates short and long-term FI [186]. Ghrelin is located predominantly in endocrine cells in the stomach [187]. Compared to other orexigenic peptides, ghrelin works against the actions of leptin [182] serving as a meal initiation signal [188]. Ghrelin concentrations follow a preprandial rise and a postprandial decline [188] similar to that of insulin. The administration of pharmacological doses of ghrelin in rodents [189] and humans [190], promotes FI and enhances weight gain [189]. Adults increased their energy intake by 28% at a meal provided 2 h after receiving a ghrelin infusion (5.0 pmol/kg/min) compared to after a saline infusion [190] supporting the role of ghrelin in stimulating FI.

Similar to other gut hormones, ghrelin's response is dependent on macronutrient

composition [61]. High carbohydrate foods have been shown to suppress plasma ghrelin concentrations [103]. In one study, OB children provided with a 0.75 g/kg glucose solution experienced a significant decrease in ghrelin levels after the oral glucose dose [191]. This indicates that plasma ghrelin levels decrease significantly after the ingestion of glucose [192]. Similarly, the ingestion of a high carbohydrate diet (60% of energy from carbohydrate) led to a significant decline in ghrelin concentrations in women [103].

The amount of circulating ghrelin is influenced by acute and long-term changes in nutritional status [193]. Fasting ghrelin concentration is inversely associated with percent body fat as well as fasting insulin and leptin concentrations [129]. Hence, plasma ghrelin levels in OB individuals tend to be lower than their lean counterparts [188]. Plasma ghrelin is also higher in patients with anorexia nervosa compared to individuals with a normal BW [194]. Furthermore, fasting plasma ghrelin levels are inversely associated with fasting plasma levels of insulin and leptin [103] which suggests that ghrelin is down-regulated in OB individuals. The up-regulation of ghrelin during negative energy balance and down-regulation during positive energy balance suggests the presence of a negative feedback mechanism which maintains energy homeostasis [188].

2.7.2. Long-Term Gastrointestinal Hormones

2.7.2.1. Leptin

Leptin, a satiety hormone secreted primarily by adipose tissue [195], regulates long-term FI [195-197] and feeding behavior [197] by suppressing subjective appetite [185]. Leptin secretion by adipocytes is directly proportionate to FM [198] and therefore reflects the body's energy stores. In humans, the secretion of leptin is stimulated by FI [199] and the release of insulin in the bloodstream which occurs shortly after meals [200]. Circulating leptin stimulates leptin hypothalamic receptors after crossing the blood-brain barrier to decrease FI and increase energy expenditure [201] which helps the body maintain appropriate fat stores [202].

The body tightly regulates energy balance and although short-term changes in energy intake can be matched to energy expenditure, long-term disparities may lead to changes in body composition [182]. Obese individuals often have elevated leptin levels and may be leptin resistant [11, 123]. When the hypothalamus is exposed to high levels of leptin for an extended period, the body becomes desensitized to the hormone resulting in elevated, sustained leptin

levels in the body. Leptin-deficient patients given small doses of leptin experience weight loss composed exclusively of body fat [203], while animals given chronic doses of leptin experience reductions in FI [204]. Leptin regulation is dependent on the efficiency of leptin receptors [205]. Genetically OB (fa/fa) Zucker rats do not have functional leptin receptors [182] and therefore secrete gastric leptin at a slower rate than lean rats [205]. Zucker rats also have elevated levels of circulating leptin [206]. This suggests that the functional ability of leptin receptors may not be required to stimulate leptin secretion [182].

2.7.2.2. Insulin

The effect of insulin on FI is based on its relationship with BG concentrations. Insulin functions as an adiposity signal in the long-term regulation of FI [207], energy homeostasis and BW [11] by stimulating glucose uptake and regulating BG levels [208]. Changes in plasma insulin are identified by the CNS leading to a decrease in FI [209] and an increase in energy expenditure [210] as a means to maintain energy homeostasis. The amount of insulin secreted is dependent on the amount of recent carbohydrate and protein consumption; dietary fat does not stimulate insulin [198]. Peak insulin response occurs 30 min after the consumption of protein and carbohydrate [63]. A study conducted by Rodin et al. (1988) found that a 50 g glucose preload resulted in a greater stimulation of insulin compared to a fructose preload which may explain the significant reduction of FI after the fructose drink [211]. It was reported in a recent met-analysis conducted by Flint et al. (2007) that insulin as opposed to glucose functions as a satiety signal in the regulation of short-term FI in NW but not OB individuals [118].

2.8. Measures of Short-Term Food Intake, Subjective Appetite, Body Composition and Dietary Restraint in Children and Adolescents

The following section will define several key terms associated with subjective appetite including hunger, satiation and satiety. In addition, this section will describe the methods and instruments used to measure short-term FI, subjective appetite, body composition and dietary restraint in children. The accuracy and validity of these methods has been demonstrated in adults, although there is less evidence surrounding their use in children.

2.8.1. Hunger, Satiation and Satiety

Hunger, satiation and satiety are important terms related to subjective appetite which is

often investigated in relation to FI regulation. Hunger is defined as the natural drive to find food [212]. Conversely, satiation [212] is the capacity of ingested foods to suppress hunger and inhibit arrival of the next eating period [213]. Satiation involves the process which controls the size of a meal by ending the eating period [214]. Satiation can be studied by providing individuals with foods varying in composition, and measuring the amount consumed when the food is freely available. Alternatively, satiety [212] is the inhibition of hunger and FI that occurs following a meal and is described as the process that terminates an eating period [71]. Satiety is associated with the length of time between meals and/or the amount of food consumed during a subsequent eating episode [214]. Both satiety and satiation influence the type and the quantity of food consumed [212] and therefore both must be considered in the context of this research.

2.8.2. Subjective Appetite

Visual analogue scales (VAS) are used to measure subjective appetite sensations. A VAS is a 100 mm line affixed with contrasting statements at both ends. Visual analogue scales are used to assess motivation to eat. The original VAS questionnaire was developed by Hill and Blundell (1982) and included the following 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); '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) [215].

Previous research has utilized VAS to assess subjective appetite in children [12, 146] and adults [118, 216]. Studies have supported the reproducibility of VAS to measure subjective appetite ratings in adults [217] and children as indicated by consistent AA ratings prior to a lunch meal [12]. A study conducted in 9-14 year old boys found that subjective appetite scores prior to a test meal were strongly correlated with FI during the meal, and post-meal subjective appetite ratings were reduced [12]. This suggests that children understand VAS questionnaires and are able to quantify their subjective hunger [12]. Among 9-10 year olds, FI led to a significant decrease in subjective DTE and hunger indicating that children are able to complete VAS in a quantitative manner [68].

2.9. Measurement of Short-Term Food Intake

A preload design is often used to investigate the effect of carbohydrate, protein and fat on short-term FI. This design provides a framework to measure the magnitude of satiety signals resulting from preloads differing in macronutrient composition [218]. In this design, subjects consume a calorie containing treatment as well as a calorie-free control [12] which is followed by a short delay and then an ad libitum test meal is provided [71, 219]. The effect of the treatment on FI is determined using CC which is a term used to explain the reduction or compensation of energy intake at a test meal following the consumption of a caloric treatment compared to a calorie-free control. CC is calculated using the following formula: CC (%) = [Control intake (kcal) – Treatment intake (kcal)/ kcal in treatment preload] by 100 [12, 68, 74, 75, 220]. Compensation scores equal to 100% suggest that energy intake at the test meal was reduced by the same number of calories contained in the caloric treatment. Compensation scores >100% indicate overcompensation or consuming more than the number of calories than that contained in the caloric treatment and scores <100% indicate undercompensation or consuming less calories than that contained in the caloric treatment [12, 68, 74, 75, 216].

2.10. Measurement of Body Composition

The most accurate method for measuring body composition is the analysis of cadavers; however there is currently no technique available to measure body composition with a similar accuracy [219]. In children, there are three methods commonly used to assess body composition, which include skinfold thickness measurements, DXA and BIA [219]. However, it is important to recognize that a single measurement technique may not be appropriate for all circumstances. The following section will describe several universal techniques for estimating FM and FFM within the pediatric age range.

2.10.1. Skinfold Measurement

Skinfold analysis is a technique used to assess subcutaneous body fat [219]. The skinfold technique is conducted by pinching subject's skin between the thumb and forefinger and placing calipers on the fold to measure the width of the two layers of skin which represents the subcutaneous fat. Several equations are used to predict percent body fat or body density from skinfold measurements [78, 84]. These equations however may not be valid in populations which are different from those in which they were derived [219]. Predictive equations often confound

raw values with predictive error (standard error of the estimate) [219]. When assessing adiposity in children, measurements should be kept as raw values or standard deviations to provide reliable measurements of regional adiposity [219].

There are several benefits of using skinfold analysis. Skinfold measurements are quick and easy to obtain and the technique is noninvasive and does not require radioactive exposure which limits the risk to subjects. The instrument is also inexpensive, does not require electrical power to operate, and the measurements can be performed anywhere by a trained technician [221]. Cross validation studies have found that skinfold estimates of FM are more strongly correlated with a 4-compartment criterion method ($r^2 = 0.62$) compared to BIA ($r^2 = 0.43$) [222]. Conversely, there are several limitations associated with skinfold analysis. First, there are structural limitations associated with Lange® skinfold calipers which have a maximum diameter of 60 mm [223]. This constraint decreases the accuracy of skinfold measures conducted in OB children. Further, the regression equations used to predict body fat from anthropometric measurements are based on observations from studies involving small sample sizes of cadavers and may not be specific to the OB pediatric population [224]. Evidence has also found that predicting FM from skinfold thickness equations has a variability of approximately 10% [221]. However, research indicates that skinfold analysis provides an accurate predictor of FM in both children and adolescents [225].

2.10.2. Dual Energy X-ray Absorptiometry

DXA is a procedure which measures body composition as well as FM [223]. This technique detects photons emitted at two different energy levels to determine bone mineral density as well as lean and soft tissue masses [226]. This method uses ionizing radiation although the effective dose is below background levels which allows the subcutaneous fat tissue to be measured [219]. DXA is a quick and acceptable method for children as young as four years of age as well as infants [219]. Although DXA provides a measurement FM and FFM, there is a lack of reference data among the pediatric population [219].

2.10.3. Bioelectrical Impedance Analysis

BIA is a simple and inexpensive method used to predict body composition [12]. This technique measures the impedance of the body to a small electrical current [219]. The generic theoretical model for BIA analyzes the body as a single cylinder using measurements made

between electrodes placed on the wrist and ankle [219]. Total body water is then estimated using regression equations as a means to determine FFM. FFM is a better conductor of electricity than fat due to its higher water content [227]. Therefore, greater amounts of FFM and hence total body water provide less resistance to the flow of electricity. Evidence suggests that BIA is a less precise method for measuring FM in young children compared to skinfold measurements [29]. In eleven, 9-14 year old boys, BIA underestimated FM by approximately 4 kg compared to skinfold analysis [228].

There are several challenges associated with the accuracy of this method. The association between bioelectrical values and total body water is influenced by the characteristics of the population being assessed [219]. Body composition is fairly consistent over short time periods in growing children, therefore BIA may be used to indicate the direction of change in FFM, but it is unable to accurately quantify the magnitude of this change [219]. Consequently, BIA may not be a reliable method for estimating body composition in children.

2.11. Summary

The prevalence of childhood obesity is on the rise and has reached epidemic proportions worldwide. Obese children are at an increased risk of becoming OB as adults and suffering from chronic diseases including cardiovascular disease, hypertension and diabetes. This increases the financial burden on the healthcare system. Previous research has focused on the environmental factors affecting obesity although in general, the physiological mechanisms affecting FI regulation are less clear. Research has failed to examine the effect of macronutrient and body composition on short-term FI regulation. A greater understanding of these factors may help prevent childhood obesity through the development of recommendations to reduce short-term FI. This research was designed to compare the effect of isovolumetric (350 ml) preloads of fruit drink, cola and 1% chocolate milk on short-term FI and subjective appetite when compared to a water control in 9-14 year old NW and OW/OB boys.

Chapter 3. Hypothesis & Objective

3.1. Hypothesis

1% chocolate milk will increase meal-time satiation and decrease short-term FI to a greater extent than other isovolumetric SSB in NW and OW/OB boys, although the effect will be diminished in OW/OB boys.

3.2. Objective

To compare the effect of isovolumetric (350 ml) preloads of fruit drink, cola and 1% chocolate milk on subjective appetite and short-term FI when compared to a water control in 9-14 year old NW and OW/OB boys.

Chapter 4. Methods

4.1. Subjects and Screening Process

Sixteen NW (between the 5th and 85th age and sex-specific BMI percentile) and sixteen OW/OB (above the 85th age and sex-specific BMI percentile) boys between the ages of 9 and 14 years were recruited to participate in this study. Boys were recruited primarily through word of mouth, flyers were posted around Halifax Regional Municipality where parents of school-age children frequent (i.e. community centers, gyms, public libraries) and advertisements were placed in the newspaper (**Appendix 9.1.**). A recruitment letter was also given to the parent/guardian of potential subjects (**Appendix 9.2.**). The study was approved by the Mount Saint Vincent University Research Ethics Board (File #: 2010-098).

Boys born at full-term within the normal birth weight range and who had maintained a stable BW for ≥ 6 months (as confirmed by the parent/guardian) were included in the study. Conversely, children who were following a restricted diet, taking medications that affected appetite or FI, had significant learning, behavioral or emotional difficulties that may have affected their ability to comprehend the VAS or who had allergies to any of the test treatments were excluded. A telephone screening questionnaire (**Appendix 9.3.**) was conducted prior to all subjects attending the physical screening/information session. If the study criterion was met, the parent and child attended a physical screening/information session at Mount Saint Vincent University in the Department of Applied Human Nutrition. During this session, the study procedures were fully explained to the parent and children and the child was introduced and familiarized with the research laboratory as well as the VAS questionnaires (**Appendix 9.8.**) to limit apprehension during the first test session. Parental informed consent (**Appendix 9.4.**) and a child's written assent (**Appendix 9.5.**) was obtained from the parent and child respectively. Physical measurements of the child's height (cm) and weight (kg) were assessed using a balance scale and skinfold measurements were taken at 4 points (triceps, biceps, supra-ilial, and subscapular) using a Lange skinfold calliper, measured to the nearest 0.1 mm and recorded on the Information Session Study Sheet (**Appendix 9.6.**). A Dutch Eating Behaviour Questionnaire was also administered to measure dietary cognitive restraint, disinhibition, and emotional eating (**Appendix 9.9.**). Subjects who had difficulty comprehending the questionnaires were provided with assistance from the investigator.

4.2. Experimental Procedure

Subjects arrived at the Department of Applied Human Nutrition at Mount Saint Vincent University 2 h after consuming a standardized breakfast of fat-free skim milk (Baxter's Skim 0% M.F.® 250 ml, 90 kcal), breakfast cereal (Honey Nut Cheerios® 26 g, 103 kcal) and Tropicana Orange Juice® (236 ml, 110 kcal) at home (**Table 4.1.**). All components of the standardized breakfast were purchased at Sobeys, Halifax, N.S. The experimental sessions remained consistent for each session and began at 10:00 am, 11:00 am or 12:00 pm. Upon subject's arrival, investigators conducted a compliance survey to ensure the child had consumed the entire standardized breakfast provided to them; the child's response was then verified with the parent(s) and recorded on the Feeding Session Cover Sheet (**Appendix 9.7.**). Subjects reporting deviations from the study's protocol or their usual patterns were asked to reschedule. Subjects were required to complete VAS questionnaires rating their motivation-to-eat and physical comfort at baseline (0 min), 15, 30, 45 and 60 min and post meal (90 min).

Preload treatments were provided to subjects in random order one week apart. The treatments were equivolumetric (350 ml) and included fruit drink (Fruite®, 154 kcal), cola (Coca Cola®, 158 kcal), 1% chocolate milk (Baxter's® 1% M.F Chocolate Milk, 224 kcal) and a water control (Nestle Pure Life®, 0 kcal) purchased at Sobeys, Halifax, N.S. (**Table 4.2.**). Treatments were prepared, stored in the refrigerator and served chilled in an opaque cup with a lid and straw. Subjects were escorted to the sensory evaluation room (Evaristus 366) and seated in individual cubicles to minimize distractions. They were then served the treatment followed by 100 ml of water to minimize aftertaste which they consumed within five minutes. Following consumption of the treatment, subjects engaged in age appropriate activities while seated for 60 min and completed VAS questionnaires at the appropriate time points. At 60 min, boys were escorted to the taste panel room, individually seated in cubicles and provided with an ad libitum pizza lunch. During the initial screening session, boys were asked to indicate which type of pizza they would prefer during the test meal. Two varieties of "Deep 'N Delicious 5" diameter pizzas were fed; pepperoni and three-cheese pizza (donated by McCain Canada Ltd., Florenceville, New Brunswick) (**Table 4.3.**). During the test meal, boys were instructed to eat until they were 'comfortably full' and were informed that additional trays of hot pizzas would be provided at regular intervals. Boys were also provided with a 500 ml bottle of water with the pizza lunch. Boys were provided with 3 cooked pizzas which were weighed and cut into four equal pieces

before serving. Fresh trays of pizza were provided to subjects at 60, 70 and 80 min for a total of 9 pizzas. The amount of pizza left after the meal was subtracted from the initial weight and converted to kilocalories to provide a measure of FI (**Appendix 9.10.**).

Table 4.1. Nutritional composition of standardized breakfast

	Honey Nut Cheerios (26 g)	Skim Milk (250 ml)	Orange Juice (236 ml)
Calories (kcal)	102	90	110
Fat (g)	1	0	0
Saturated Fat (g)	0	0	-
Trans Fat (g)	0	0	-
Cholesterol (mg)	0	5	-
Sodium (mg)	149	125	0
Carbohydrate (g)	20	13	27
Sugar (g)	8	13	23
Fiber (g)	2	0	-
Protein (g)	2	9	2

4.2.1. Test Treatments

Test treatments were provided to subjects in random order over a four week period. The treatments were equivolumetric (350 ml) and included water (0 kcal), fruit drink (154 kcal), cola (158 kcal) and 1% chocolate milk (224 kcal) (Sobeys, Halifax, N.S. (**Table 4.2.**). Treatments were prepared, stored in the refrigerator and served chilled in an opaque cup with a lid and straw. Subjects had approximately five minutes to consume the treatment which was followed by 100 ml of water to minimize aftertaste. The pH of each treatment was determined by taking the average pH of three consecutive measurements of the same treatment using a Fisher Scientific accumet* AB15 Basic and BioBasic pH/mV/°C Met. All treatments were 6.5°C at the time of measurement. Analytical sugars composition of the fruit drink and cola treatments was conducted by Maxaam Analytics International Corporation, Mississauga Ontario using reference method AOAC 980 [229].

Table 4.2. Nutritional composition of test treatments

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

Nutrititional composition of the treatments was conducted by Maxaam Analytics International Corporation, Missassaga Ontario.

4.2.2. Test Lunch

Two varieties of McCain Foods: Deep and Delicious 5" Pizza; pepperoni and three-cheese pizza were available for consumption. Subjects choose the type of pizza according to their preference before the first experimental session and were served the same variety of pizza during each of the sessions. The 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 per pizza. The three-cheese pizzas (81 g) contained 9 g of protein, 6 g of fat and 22 g of carbohydrate for a total energy content of 180 kcal (**Table 4.3.**). An advantage of using these pizzas was the lack of crust resulting in a uniform energy and macronutrient content. The lack of crust also eliminated the possibility of subjects eating the denser pizza filling and leaving the outside pizza crust.

Each subject was provided with three cooked pizzas per tray. The first tray of pizzas was removed after 10 min and subjects were provided with a second warm tray containing 3 more pizzas which was repeated again at 20 min. A total of 9 pizzas were served over the 30 min time period. Pizzas were weighed before serving and the amount left after the meal was subtracted from the initial weight to provide a measure of FI. The energy consumed by each subject was calculated by converting the net pizza weight consumed in grams into kilocalories using the manufacturer's nutrition information (**Table 4.3.**). Water intake was also measured by weighing the bottled water before and after the test meal to determine consumption.

Table 4.3. Nutritional composition of pizzas served at test meal

Per 1 Pizza	Pepperoni (87 g)	3-Cheese (81 g)
Calories (kcal)	180	180
Fat (g)	6	6
Saturated Fat (g)	2.5	2.5
Trans Fat (g)	0.1	0.1
Cholesterol (mg)	15	15
Sodium (mg)	400	360
Carbohydrates (g)	23	22
Fiber (g)	2	2
Sugar (g)	4	4
Protein (g)	9	9

4.2.3. Subjective Appetite

Visual analogue scale questionnaires assessed AA and were composed of four questions. Subject's motivation to eat was measured using the following questions: (1) 'How strong is your desire to eat?' ("very weak" to "very strong"), (2) 'How hungry do you feel?' ("not hungry at all" to "as hungry as I've ever felt"), (3) 'How do you feel?' ("not full at all" to "very full") and (4) 'How much food do you think you could eat?' (prospective food consumption; "nothing at all" to "a large amount"). The subjective sweetness of the preload treatments was measured by asking subjects, 'How sweet have you found the beverage?' ("not sweet at all" to "very sweet") and the subjective pleasantness of the preload treatments and test meal were measured by asking 'How pleasant have you found the preload/food?' ("not at all pleasant" to "very pleasant"). Subjective appetite scores were determined by measuring the distance in mm from the left starting point of the line to the intersection of the "x". AA scores were calculated at each time point, for each treatment consumed by each subject using the formula: AA score (mm) = [desire to eat + hunger + (100 – fullness) + PFC] / 4 which incorporates the 4 questions from the motivation-to-eat VAS [47, 68, 74, 80, 90].

4.2.4. Estimation of Body Composition

Skinfold measurements were obtained during the initial screening session. Skinfold analysis using a Lange skinfold caliper (Beta Technology Incorporated-Cambridge, Maryland) was used to measure skinfold thickness at four sites (triceps, biceps, supra-ileal, and subscapular). Measurements were recorded to the nearest 0.1 millimeter. The mean skinfold measurements at each site were used to estimate FM for each subject. Body density was calculated using a sex-specific regression equation (**Equation 1**) and percent body fat was estimated **(Equation 2)** [84].

Equation 1: Density = 1.1690 – 0.0788 * log (sum of skinfold thicknesses at 4 sites)

Equation 2: Body fat percentage of body weight = [(4.95/density brook) - 4.5]

Subject's weight classification including their BMI and BMI percentile was calculated using the Centers for Disease Control and Prevention online BMI Percentile Calculator for Children and Teens [230]. This calculator incorporated each subjects' date of birth, date of measurement, sex, height and BW to determine their BMI percentile.

4.2.5. Determination of Dietary Cognitive Restraint

The Dutch Eating Behaviour Questionnaire (DEBQ) was developed in 1986 by van Strein, Frijters, Bergers and Defares [120]. The DEBQ is a 33-item inventory used to measure dietary restraint and disinhibition. The questionnaire consists of three factors: (1) dietary restraint, or cognitive control of eating, (2) emotional disinhibition, or loss of control of eating due to emotions and (3) external disinhibition, or loss of cognitive control of eating due to the presence of food. Emotional disinhibition is divided into two subcategories: (a) loss of cognitive control of eating due to specific emotions (i.e. eating caused by emotions resulting from a recent event) or (b) loss of cognitive control of eating due to diffused emotions (i.e. eating caused by boredom). Dietary disinhibition is defined as a combination of emotional and external disinhibition.

The DEBQ has been used in a variety of studies to assess children's eating styles [74] and is a reliable and valid tool to assess the various types of eating behaviours [184]. There are three types of eating behaviours hypothesized to be associated with excessive snacking, weight gain and bingeing. These behaviours include; emotional eating or eating in response to negative emotion, external eating or eating in response to the sight or smell of food and dietary restraint, which involves eating less than what is desired to either lose or maintain BW [231]. The DEBQ was used in this study to verify the presence of restrained, emotional and disinhibited eating which may provide a potential explanation for differences in FI.

4.3. Ethical Considerations

Ethics approval for this study was obtained from the Mount Saint Vincent University Research Ethics Board (UREB File # 2009-020). Parental consent and child's assent for participation was obtained at the initial screening session before beginning experimental sessions and all subjects were given a code and a number to identify them in all documents, records and files. All data pertaining to the study was entered into Microsoft Excel files and was available to the primary investigators. All records relating to subjects were kept confidential in a locked cabinet in the Department of Applied Human Nutrition (Evaristus 366) at Mount Saint Vincent University. No disclosure of personal information of subjects or parents/guardians took place except if required by law. All documents pertaining to the study will be kept for a minimum of five years

following completion of the study and will then be securely destroyed.

4.4. Statistical Analysis

SAS version 9.2 (Statistical Analysis Systems, SAS Institute Inc., Carey, NC) was used to perform all statistical analyses and all data are reported as mean ± SEM (standard error of the mean). Subject's baseline characteristics were analyzed between groups by Student's independent samples t-test. FI, treatment dose, water intake, CC, sweetness and pleasantness of the treatments and pleasantness of the test meal were analyzed using the 2-factor Mixed Model procedure with treatment and weight status as main factors. Post-hoc analysis of statistically significant differences was completed using Tukey-Kramer's test, adjusted for multiple comparisons when main effects or interactions were statistically significant which was indicated by a P-value < 0.05.

A 3-factor Mixed Model procedure was used to determine the affect of treatment, weight status and time on subjective appetite scores including: AA, DTE, hunger, fullness and PFC, physical comfort and thirst. Subjective appetite scores are reported as absolute values. Change from baseline subjective appetite scores were also reported to correct for differences in subjective feelings of appetite upon arrival to the test sessions at 15, 30, 45 and 60 min. Change from baseline scores were also adjusted for the energy content of the preloads to correct for differences in the caloric contents of the treatments. Change from baseline per kcal scores were calculated by dividing the change from baseline scores by the number of calories contained in the corresponding treatment at each time point.

Pearson correlation coefficients were reported to assess associations among dependent measures including; absolute subjective appetite scores (AA, DTE, hunger, fullness, PFC, thirst), preload sweetness, body composition (BW, FM, FFM), dietary restraint, disinhibition and emotional eating and treatment dose (kcal/kg BW).

AA scores were calculated at each time point for each treatment using the formula:

AA score (mm) = [DTE + hunger + (100-fullness) + PFC]/4 which reflects the 4 questions on the motivation to eat VAS [12, 47, 146, 228, 232, 233].

CC scores were also calculated for each subject after each treatment using the formula:

CC (%) = [control intake (kcal) – treatment intake (kcal)/ kcal in preload treatment] x 100 [12, 47, 146, 228, 232, 233].

Chapter 5. Results

5.1. Subjects

Sixteen NW boys (mean ± SEM; 12.1 ± 0.4 y) with a mean BMI percentile of 50.2 ± 7.1 and sixteen OW/OB boys (mean ± SEM; 11.6 ± 0.5 y) with a mean BMI percentile of 94.2 ± 1.0 were included in this study (**Table 5.1.**).

Table 5.1. Subject baseline characteristics

Subject Characteristics	NW	OW/OB
Age (y)	12.1 ± 0.4	11.6 ± 0.5
Body Weight (kg)	43.3 ± 2.3	57.3 ± 3.6*
Height (m)	1.6 ± 0.03	1.5 ± 0.03
BMI (kg/m^2)	17.4 ± 0.8	25.3 ± 1.0*
BMI Percentile	50.2 ± 7.1	94.2 ± 1.0*
Fat Mass (kg)[1]	6.9 ± 1.0	13.4 ± 2.2
Fat Mass (%)[1]	16.1 ± 2.3	22.8 ± 2.9*
Fat-Free Mass (kg) [1]	36.4 ± 2.2	43.9 ± 3.0
Fat-Free Mass (%)[1]	83.9 ± 2.3	77.2 ± 2.9
DEBQ Average Score[2]	1.8 ± 0.1	2.1 ± 0.2
Restraint[2]	1.8 ± 0.1	2.1 ± 0.2
Overall Disinhibition[2]	1.8 ± 0.2	1.4 ± 0.1
Diffuse Emotional Disinhibition[2]	4.6 ± 0.5	6.6 ± 0.6*
Specific Emotional Disinhibition[2]	1.7 ± 0.2	1.4 ± 0.1
External Disinhibition[2]	2.9 ± 0.1	2.7 ± 0.2

Data are presented as mean ± SEM, n=16 NW, n=16 OW/OB. Abbreviations: BMI, body mass index; NW, normal weight; OW, overweight; OB, obese; DEBQ, Dutch Eating Behavior Questionnaire. [1]FM and FFM were determined from the sum of skinfold measurements at four points. [2]Dietary restraint and disinhibition were assessed using the DEBQ [120]. *Significantly different by Student's independent samples t-test ($P < 0.05$).

5.1.1. Food Intake (kcal)

FI at the test meal was affected by treatment ($P<0.0001$) but weight status was not a factor ($P=0.48$) and there was no significant treatment x weight interaction ($P=0.66$). FI was reduced after 1% chocolate milk ($P<0.0001$) and cola ($P=0.02$) compared to the water control, and after 1% chocolate milk compared to the fruit drink ($P=0.005$) (**Table 5.2.**).

5.1.2. Cumulative Food Intake, Test Meal and Treatment (kcal)

Cumulative FI (test meal + treatment) was affected by treatment ($P=0.03$) but weight status was not a factor ($P=0.48$) and there was no significant treatment x weight interaction ($P=0.66$). Boys consumed more energy after the fruit drink compared to the water control ($P=0.02$) (**Table 5.2.**).

5.1.3. Food Intake, Test Meal (kcal/kg BW)

FI at the test meal, expressed relative to the BW of subjects, was affected by treatment ($P<0.0001$) and weight status ($P=0.02$) but there was no treatment x weight interaction ($P=0.46$). OW/OB boys consumed significantly less energy per kg BW at the test meal compared to NW boys ($P=0.02$). Boys consumed less energy per kg BW after cola ($P=0.01$) and 1% chocolate milk ($P=0.0001$) compared to the water control, and after 1% chocolate milk compared to the fruit drink ($P=0.02$) (**Table 5.2.**).

5.1.4. Treatment Dose (kcal/kg BW)

Treatment dose expressed relative to the BW of subjects, was affected by treatment ($P<0.0001$) and weight status ($P<0.01$). NW boys received larger treatment doses compared to OW/OB boys ($P=0.002$). Boys received more energy per kg BW from 1% chocolate milk compared to the fruit drink and cola treatments ($P<0.0001$). There was a significant treatment x weight interaction ($P<0.0001$) as OW/OB boys received a smaller treatment dose per kg BW of the 1% chocolate milk treatment compared to NW boys ($P=0.002$) (**Table 5.2.**).

5.1.5. Water Intake (g)

Treatment ($P=0.08$) nor weight status ($P=0.60$) affected water intake at the test meal and there was no treatment x weight interaction ($P=0.07$) (**Table 5.2.**).

5.1.6. Caloric Compensation

Treatment (P=0.11) nor weight status (P=0.27) affected CC and there was no treatment x weight interaction (P=0.79). NW boys had CC scores of 44%, 96% and 90% after the fruit drink, cola and 1% chocolate milk treatment, respectively. OW/OB boys had CC scores of 16%, 40% and 61% after the fruit drink, cola and 1% chocolate milk treatments respectively (**Table 5.2.**). When the sample data was pooled, treatment was not a factor affecting CC (P=0.11).

5.1.7. Sweetness of Preload Treatments

Preload sweetness was affected by treatment (P=0.02) but not weight status (P=0.55) and there was no treatment x weight interaction (P=0.62). Subjective sweetness of the fruit drink was significantly higher compared to cola and 1% chocolate milk (**Table 5.2.**).

5.1.8. Pleasantness of Preload Treatments

Preload pleasantness was affected by treatment (P=0.005) and weight status (P=0.03) but there was no treatment x weight interaction (P=0.05). NW boys rated the treatments as more pleasant than OW/OB boys (P=0.03). Subjective pleasantness of 1% chocolate milk was significantly higher compared to cola (P=0.001) (**Table 5.2.**)

5.1.9. Pleasantness of Test Meal

Subjective pleasantness of the test meal was not affected by treatment (P=0.07) or weight status (P=0.85) and there was no weight x treatment interaction (P=0.08) (**Table 5.2.**).

Table 5.2. Effect of preload treatments on food intake 60 min later in NW and OW/OB boys

	NW				OW/OB			
	Water	**Fruit Drink**	**Cola**	**1% Chocolate Milk**	**Water**	**Fruit Drink**	**Cola**	**1% Chocolate Milk**
FI[1], kcal	1046 ± 51	984 ± 64	894 ± 54†	844 ± 52†€	1050 ± 49	1026 ± 63	986 ± 75	912 ± 63
Cumulative FI[2] (test meal + preload), kcal	1046 ± 51	1138 ± 64†	1052 ± 54	1068 ± 52	1050 ± 49	1180 ± 63	1144 ± 75	1136 ± 63
FI[1], kcal/kg BW	25 ± 2	24 ± 2	21 ± 2†	21 ± 2†€	19 ± 1	19 ± 1	18 ± 1	17 ± 1
Treatment Dose, kcal/kg BW	-	3.73 ± 0.22	3.84 ± 0.22	5.43 ± 0.31µ	-	2.84 ± 0.16	2.91 ± 0.17	4.13 ± 0.24
Water Intake (g)	176 ± 44	214 ± 46	218 ± 44	269 ± 41	225 ± 49	285 ± 48	261 ± 47	230 ± 42
CC[3], %	-	44 ± 34	96 ± 31	90 ± 17	-	16 ± 35	40 ± 39	61 ± 16
Sweetness, mm	-	81 ± 5§	73 ± 6	69 ± 4	-	79 ± 6	63 ± 8	67 ± 7
Pleasantness, mm	-	80 ± 6	80 ± 7	85 ± 4¥	-	66 ± 7	53 ± 8	87 ± 4
Test Meal Pleasantness, mm	86 ± 3	89 ± 3	82 ± 6	84 ± 4	88 ± 5	89 ± 4	91 ± 3	77 ± 6

Treatment effects were analyzed using the PROC MIXED procedure with treatment and weight status as main factors. Data are presented as means ± SEM; n=16 NW and n=16 OW/OB. Abbreviations: CC, caloric compensation; NW, normal weight; OW, overweight; OB, obese. † $p < 0.05$ versus NW and OW/OB water; € $p < 0.05$ versus NW and OW/OB fruit drink; µ $p < 0.05$ versus OW/OB 1% chocolate milk; § $p < 0.05$ versus NW and OW/OB cola and 1% chocolate milk; ¥ $p < 0.05$ versus NW and OW/OB cola. [1]Pizza intake at test meal. [2]Cumulative energy intake from test meal and preload (kcal). [3] CC (%) = [control intake (kcal) – treatment intake (kcal)/ kcal in treatment preload] x 100.

5.2. Subjective Average Appetite Scores

5.2.1. Average Appetite

Absolute average appetite (AA) was affected by weight status ($P<0.0001$), treatment ($P=0.004$) and time ($P<0.0001$) but there were no significant interactions among weight status, treatment or time. AA was higher in NW compared to OW/OB boys ($P<0.0001$). AA was significantly higher after cola compared to the fruit drink ($P<0.0001$) and AA increased over time for all treatments (**Figure 5.1a**).

Change from baseline AA was not affected by weight status ($P=0.06$) or treatment ($P=0.24$) but time was a factor ($P<0.0001$) and there were no significant interactions among weight status, treatment or time (**Figure 5.2a**).

AA reported as the change from baseline corrected for the energy content of the treatments was not affected by weight status ($P=0.32$) or treatment ($P=0.10$) but time was a factor ($P<0.0001$) and there were no significant interactions among weight status, treatment or time (**Figure 5.3a**).

5.2.2. Desire to Eat

Absolute desire to eat (DTE) was affected by weight status ($P<0.0001$) and time ($P<0.0001$) but not by treatment ($P=0.27$) and there were no significant interactions among weight status, treatment or time. DTE was higher in NW compared to OW/OB boys ($P<0.0001$) and DTE increased over time for all treatments (**Figure 5.1b**).

Change from baseline DTE was not affected by weight status ($P=0.92$) or treatment ($P=0.66$) but time was a factor ($P<0.0001$) and there were no significant interactions among weight status, treatment or time (**Figure 5.2b**).

DTE reported as the change from baseline corrected for the energy content of the treatments was not affected by weight status ($P=0.65$) or treatment ($P=0.94$) but by time ($P<0.0001$) and there were no significant interactions among weight status, treatment or time (**Figure 5.3b**).

5.2.3. Hunger

Absolute hunger was affected by weight status ($P<0.0001$), treatment ($P=0.003$) and time ($P=0.0001$) but there were no significant interactions among weight status, treatment or time. Hunger was higher in NW compared to OW/OB boys ($P<0.0001$). Boys were significantly more hungry after cola compared to the fruit drink ($P=0.02$) and hunger increased over time for all

treatments (**Figure 5.1c**).

Change from baseline hunger scores were not affected by weight status (P=0.68) or treatment (P=0.51) but by time (P=0.0001) and there were no significant interactions among weight status, treatment or time (**Figure 5.2c**).

Hunger reported as the change from baseline corrected for the energy content of the treatments was not affected by weight status (P=0.69) or treatment (P=0.29) but by time (P<0.0001) and there were no significant interactions among weight status, treatment or time (**Figure 5.3c**).

5.2.4. Fullness

Absolute fullness was affected by weight status (P=0.0004), treatment (P=0.04) and time (P=0.008). Fullness was higher in OW/OB compared to NW boys (P=0.0004). Boys were significantly more full after the fruit drink compared to the water control (P=0.03) and fullness decreased over time for all treatments. There was a significant treatment x weight interaction (P=0.03), as fullness was significantly higher after the fruit drink (P=0.02) and water control (P=0.004) in OW/OB boys compared to NW boys (**Figure 5.1d**).

Change from baseline fullness was affected by weight status (P=0.002), treatment (P=0.008) and time (P=0.006). There was a greater decrease in fullness in OW/OB compared to NW boys (P=0.002) and there was a greater decrease in fullness after the fruit drink (P=0.04) and water control (P=0.003) compared to cola. There was a significant treatment x weight interaction (P=0.02), as the water control resulted in a significantly greater decrease in fullness in OW/OB boys, compared to NW boys (P=0.009) (**Figure 5.2d**).

Fullness reported as the change from baseline corrected for the energy content of the treatments was affected by treatment (P=0.03) and time (P=0.02) but not weight status (P=0.08). There were no significant interactions among weight status, treatment or time. Boys were significantly more full after cola compared to the fruit drink (P=0.02) (**Figure 5.3d**).

5.2.5. Prospective Food Consumption

Absolute prospective food consumption (PFC) was affected by weight status (P<0.0001), treatment (P=0.0008) and time (P=0.0001). PFC was higher in NW compared to OW/OB boys (P<0.0001). PFC was significantly higher after cola (P=0.01) and the water control (P=0.0004) compared to the fruit drink and PFC increased over time for all treatments. There was a significant treatment x weight interaction (P=0.04), as PFC was significantly higher after the

water control (P<0.0001), fruit drink (P<0.0001) and 1% chocolate milk (P=0.002) in NW boys, compared to OW/OB boys (**Figure 5.1e**).

Change from baseline PFC scores were affected by weight status (P=0.03) and time (P<0.0001) but not by treatment (P=0.17). There was a greater increase in PFC in OW/OB compared to NW boys (P=0.03). There was also a significant treatment x weight interaction (P<0.05), as the water control (P=0.003) and fruit drink (P=0.03) resulted in a significantly greater increase in PFC in OW/OB boys, compared to NW boys (**Figure 5.2e**).

PFC reported as the change from baseline corrected for the energy content of the treatment was affected by treatment (P=0.01) and time (P<0.0001) but not by weight status (P=0.25) and there were no significant interactions among weight status, treatment or time. Boys had significantly lower PFC scores after 1% chocolate milk compared to the fruit drink (P=0.009) (**Figure 5.3e**).

5.2.6. Physical Comfort

Absolute physical comfort was affected by weight status (P=0.01) but not treatment (P=0.12) or time (P=0.86). Physical comfort was higher in NW compared to OW/OB boys (P=0.01). There was a significant treatment x weight interaction (P=0.01), as physical comfort was significantly higher after 1% chocolate milk in NW boys, compared to OW/OB boys (P=0.02).

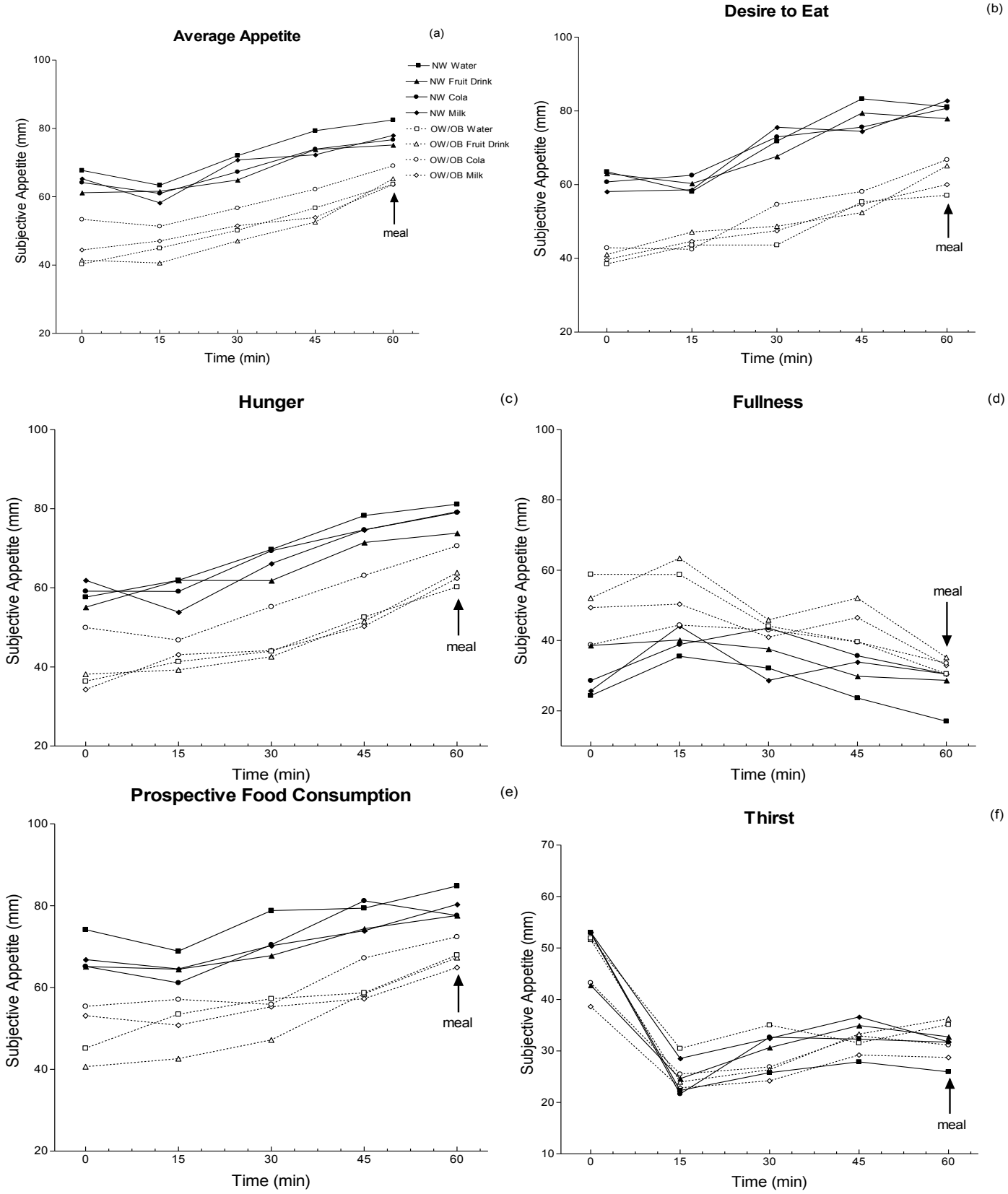
Change from baseline physical comfort scores were affected by treatment (P=0.008), but not by weight status (P=0.81) or time (P=0.82) and there were no significant interactions among weight status, treatment or time. The fruit drink (P=0.001) and cola (P=0.0003) resulted in a greater increase in physical comfort compared to 1% chocolate milk.

5.2.7. Thirst

Absolute thirst was not affected by weight status (P=0.86) or treatment (P=0.93) but by time (P<0.0001). There was a significant treatment x weight interaction (P=0.01), as thirst was significantly higher after the water control compared to 1% chocolate milk in OW/OB boys (P<0.05). Thirst scores decreased from 0 to 15 min and increased from 15 to 60 min for all treatments (**Figure 5.1f**).

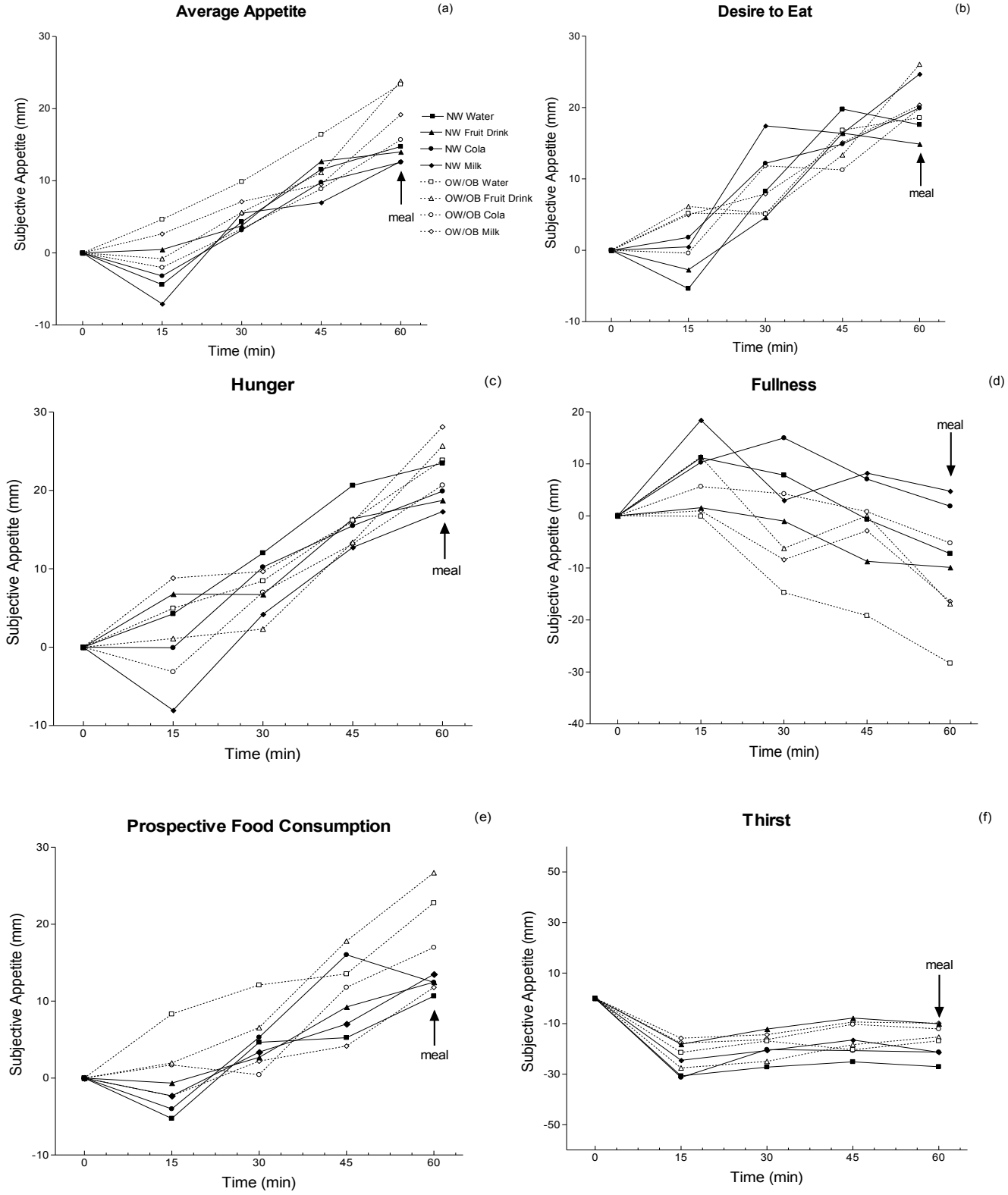
Change from baseline thirst scores were affected by treatment (P=0.02) but not by weight status (P=0.33) or time (P=0.51). The water control resulted in a greater increase in thirst compared to the fruit drink (P<0.05) (**Figure 5.2f**).

Figure 5.1. Absolute subjective average appetite after preload treatments to 60 min



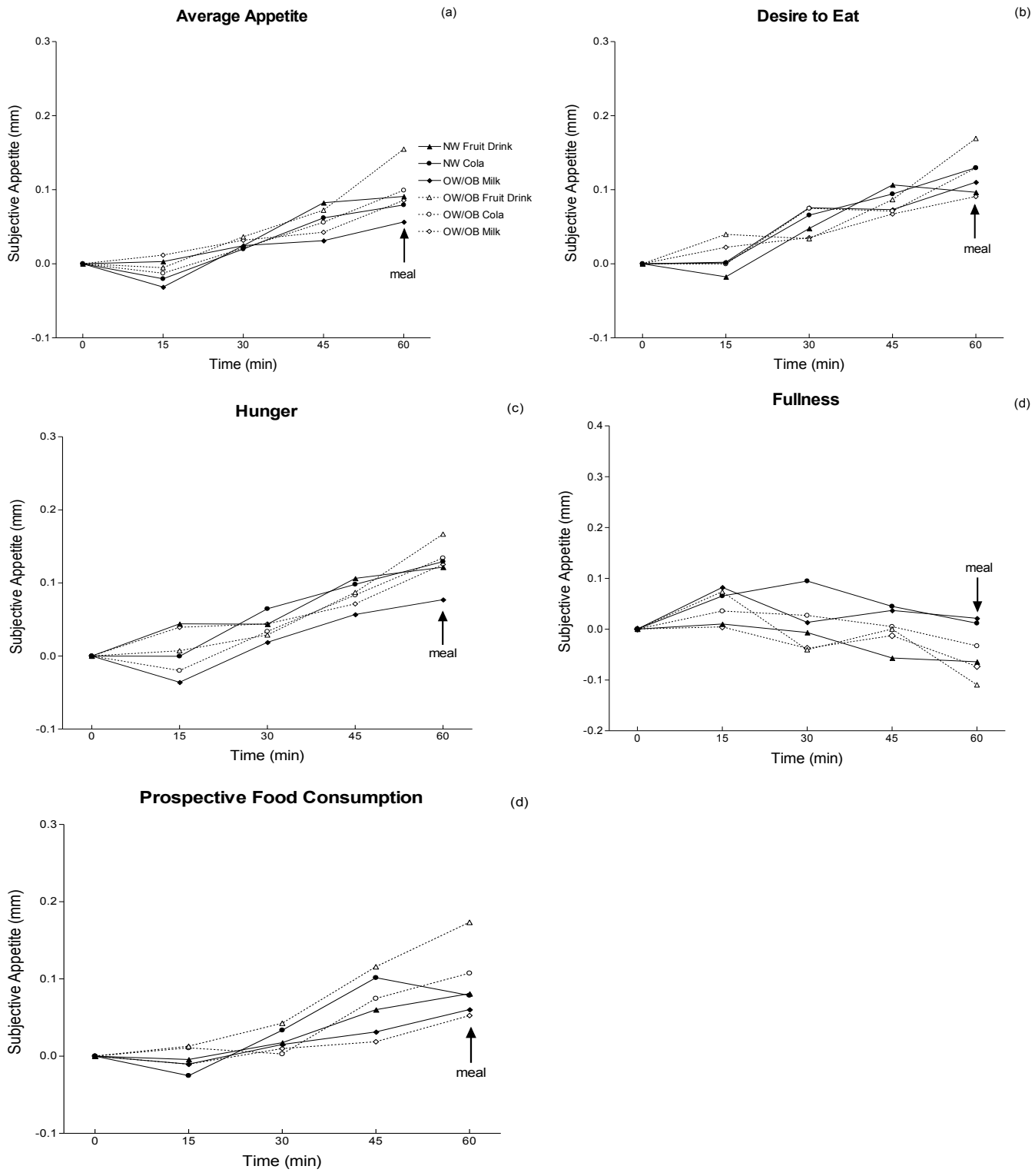
Appetite ratings for (a) AA, (b) DTE, (c) hunger, (d) fullness (e) PFC and (f) thirst at 0, 15, 30, 45 and at 60 min after consumption of water, fruit drink, cola and 1% chocolate milk treatments. Values are means, n=16 per group. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time ($P<0.001$).

Figure 5.2. Change from baseline average appetite after preload treatments to 60 min



Change from baseline appetite ratings for (a) AA, (b) DTE, (c) hunger, (d) fullness, (e) PFC and (f) thirst at 0, 15, 30, 45 and 60 min after consumption of water, fruit drink, cola and 1% chocolate milk treatments. Values are means, n=16 per group. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time ($P<0.001$).

Figure 5.3. Change from baseline per kilocalorie average appetite after preload treatments to 60 min



Change from baseline appetite ratings per kilocalorie for (a) AA, (b) DTE, (c) hunger, (d) fullness and (e) PFC at 0, 15, 30, 45 and 60 min after consumption of the control, fruit drink, cola and 1% chocolate milk treatments. Values are means, n=16 per group. NW, normal weight; OW/OB, overweight/obese. Average and individual appetite scores changed over time ($P<0.001$).

5.3. Correlations with Food Intake

5.3.1. Average Appetite

In NW boys, AA scores were positively associated with FI after the fruit drink at baseline (r=0.81, P=0.0001) and 30 min (r=0.60, P=0.01) and after 1% chocolate milk at 45 min (r=0.49, P=0.19). In OW/OB boys, AA scores were positively associated with FI after 1% chocolate milk at 15 min (r=0.56, P=0.02), 30 min (r=0.62, P=0.01), 45 min (r=0.69, P=0.003) and 60 min (r=0.73, P=0.001) (**Table 5.3.**).

Table 5.3. Associations between average appetite scores and food intake in NW and OW/OB boys

Time	0	15	30	45	60
NW					
Control	0.36	-0.21	-0.25	0.10	0.16
Fruit Drink	0.81**	0.42	0.60*	0.49	0.49
Cola	0.05	0.14	-0.09	-0.19	-0.05
1% Chocolate Milk	0.10	0.19	0.30	0.49*	0.31
OW/OB					
Control	0.27	0.47	0.40	0.49	0.35
Fruit Drink	-0.03	0.45	0.38	0.38	0.39
Cola	0.22	0.34	0.49	0.48	0.27
1% Chocolate Milk	0.50	0.56*	0.62*	0.69**	0.73**

Pearson correlation coefficients; n=16 NW, n=16 OW/OB. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05, **P < 0.01.

5.3.2. Desire to Eat

In NW boys, DTE scores were positively associated with FI after the fruit drink at 60 min (r=0.53, P=0.03). In OW/OB boys, DTE scores were positively associated with FI after 1% chocolate milk at 30 min (r=0.51, P=0.04), 45 min (r=0.61, P=0.01) and 60 min (r=0.55, P=0.03) (**Table 5.4.**).

Table 5.4. Associations between desire to eat scores and food intake in NW and OW/OB boys

Time	0	15	30	45	60
NW					
Control	0.06	-0.20	-0.24	0.27	-0.01
Fruit Drink	0.64	0.24	0.40	0.10	0.53*
Cola	0.23	0.26	0.18	-0.09	0.07
1% Chocolate Milk	0.02	0.31	0.38	0.22	0.38
OW/OB					
Control	-0.13	0.14	0.10	0.08	0.08
Fruit Drink	0.01	0.42	0.27	0.40	0.36
Cola	-0.01	0.21	0.19	0.48	0.27
1% Chocolate Milk	0.33	0.45	0.51*	0.61*	0.55*

Pearson correlation coefficients; n=16 NW, n=16 OW/OB. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05.

5.3.3. Hunger

In NW boys, hunger scores were positively associated with FI after the fruit drink at baseline (r=0.72, p=0.001), 30 min (r=0.53, P=0.04), 45 min (r=0.58, P=0.02) and 60 min (r=0.55, P=0.03). In OW/OB boys, hunger scores were positively associated with FI after cola at 45 min (r=0.55, P=0.03); and after 1% chocolate milk at 45 min (r=0.57, P=0.02) and 60 min (r=0.67, P=0.005) (**Table 5.5.**).

Table 5.5. Associations between hunger scores and food intake in NW and OW/OB boys

Time	0	15	30	45	60
NW					
Control	0.25	-0.18	-0.25	0.13	0.29
Fruit Drink	0.72**	0.01	0.53*	0.58*	0.55*
Cola	0.04	0.20	-0.13	-0.24	-0.11
1% Chocolate Milk	0.20	0.19	0.28	0.29	0.48
OW/OB					
Control	0.04	0.23	0.13	0.10	0.17
Fruit Drink	0.001	0.50	0.44	0.43	0.46
Cola	0.41	0.39	0.51	0.55*	0.30
1% Chocolate Milk	0.43	0.57	0.43	0.57*	0.67**

Pearson correlation coefficients; n=16 NW, n=16 OW/OB. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05, **P < 0.01.

5.3.4. Fullness

In NW boys, fullness scores were inversely associated with FI after the fruit drink at baseline (r=-0.65, P=0.01), 15 min (r=-0.63, P=0.01) and 30 min (r=-0.67, P=0.004). In OW/OB boys, fullness scores were inversely associated with FI after the water control at 30 min (r=-0.54, P=0.03) and 45 min (r=-0.57, P=0.02) and after 1% chocolate milk at 30 min (r=-0.63, P=0.01), 45 min (r=-0.58, P=0.02) and 60 min (r=-0.57, P=0.02) (**Table 5.6.**).

Table 5.6. Associations between fullness scores and food intake in NW and OW/OB boys

Time	0	15	30	45	60
NW					
Control	-0.36	0.08	0.20	-0.08	-0.22
Fruit Drink	-0.65*	-0.63*	-0.67**	-0.41	-0.41
Cola	0.07	0.004	0.17	0.16	0.11
1% Chocolate Milk	-0.10	-0.08	-0.21	-0.16	-0.07
OW/OB					
Control	-0.36	-0.46	-0.54*	-0.57*	-0.29
Fruit Drink	0.39	-0.10	-0.11	-0.10	-0.06
Cola	0.11	-0.34	-0.45	-0.22	-0.06
1% Chocolate Milk	-0.50	-0.44	-0.63*	-0.58*	-0.57*

Pearson correlation coefficients; n=16 NW, n=16 OW/OB. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05, **P < 0.01.

5.3.5. Prospective Food Consumption

In NW boys, PFC was positively associated with FI after the fruit drink at baseline, (r=0.74, P=0.001), 15 min (r=0.61, P=0.01), 30 min (r=0.60, P=0.01), 45 min (r=0.53, P=0.04) and 60 min (r=0.59, P=0.02). In OW/OB boys, PFC was positively associated with FI after the water control at baseline (r=0.52, P=0.04), 15 min (r=0.69, P=0.003), 30 min (r=0.62, P=0.01) 45 min (r=0.84, P<0.0001) and 60 min (r=0.72, P=0.002); with FI after the fruit drink at 15 min (r=0.59, P=0.02) and 60 min (r=0.54, P=0.03); with FI after cola at baseline, (r=0.57, P=0.02) and 30 min (r=0.61, P=0.01); and with FI after 1% chocolate milk at 15 min (r=0.56, P=0.02), 30 min (r=0.63, P=0.01), 45 min (r=0.79, P=0.0003) and 60 min (r=0.82, P=0.0001) (**Table 5.7.**).

Table 5.7. Associations between prospective food consumption scores and food intake in NW and OW/OB boys

Time	0	15	30	45	60
NW					
Control	-0.05	-0.27	-0.24	-0.15	0.08
Fruit Drink	0.74**	0.61*	0.60*	0.53*	0.59*
Cola	-0.13	0.08	-0.05	-0.19	-0.02
1% Chocolate Milk	-0.01	0.01	0.16	-0.15	0.30
OW/OB					
Control	0.52*	0.69**	0.62*	0.84**	0.72**
Fruit Drink	0.23	0.59*	0.44	0.42	0.54*
Cola	0.57*	0.28	0.61*	0.42	0.38
1% Chocolate Milk	0.44	0.56*	0.63*	0.79**	0.82**

Pearson correlation coefficients; n=16 NW, n=16 OW/OB. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05, **P < 0.01.

5.3.6. Thirst

In NW boys, thirst was not associated with FI after any of the treatments. In OW/OB boys, thirst was positively associated with FI after the fruit drink at 30 min ($r=0.59$, $P=0.02$) and with FI after cola at 15 min ($r=0.53$, $P=0.03$).

5.3.7. Preload Sweetness

When subjective sweetness ratings for each of the three sugars-sweetened preloads were correlated with FI, sweetness was positively associated with FI after cola in NW boys (r=0.53, P=0.04). There were no other significant associations between preload sweetness and FI in OW/OB boys or in the pooled sample (**Table 5.8.**).

A mean sweetness rating and a mean FI value was also calculated for each subject using the sweetness ratings and FI values after the three sugars-sweetened preloads. These mean sweetness ratings were then combined and correlated with the mean FI values for all subjects. Sweetness was not associated with FI in NW boys (r=0.44, P=0.09), OW/OB boys (r=0.21, P=0.43) or in the pooled sample (r=0.26, p=0.15) (**Table 5.9.**).

Table 5.8. Associations between preload sweetness and food intake in NW and OW/OB boys

	Fruit Drink	Cola	1% Chocolate Milk
NW	0.12	0.53*	0.25
OW/OB	0.25	0.21	0.21
All subjects	0.18	0.28	0.22

Pearson correlation coefficients; n=16 NW, n=16 OW/OB, n=32 all subjects. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05.

Table 5.9. Associations between average preload sweetness and food intake in NW and OW/OB boys

	All Treatments
NW	0.44
OW/OB	0.21
All Subjects	0.26

Pearson correlation coefficients; n=16 NW, n=16 OW/OB, n=32 all subjects. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05.

5.3.8. Body Composition

In NW boys, FFM was inversely associated with CC after cola (r=-0.55, P=0.03). In OW/OB boys, BW (r=0.53, P=0.03) and FFM (r=0.51, P=0.04) were positively associated with FI after cola. BW was also inversely associated with CC after cola (r=-0.59, P=0.02). In all subjects, BW was inversely associated with CC after the fruit drink (r=-0.36, P=0.04) and CC after cola (r=-0.56, P=0.008) and positively associated with FI after cola (r=0.46, P=0.009); FM was inversely associated with CC after cola (r=-0.36, 0.04) and FFM was positively associated with FI after cola (r=0.45, P<0.01) and inversely associated with CC after cola (r=-0.47, P=0.007) (**Table 5.10.**).

Table 5.10. Associations between body composition and measurements of food intake regulation in NW and OW/OB boys

		BW	FM[1]	FFM[1]
NW	FI Control	-0.22	0.07	-0.26
	FI Fruit Drink	0.11	0.17	0.04
	CC[2] Fruit Drink	-0.34	-0.12	-0.30
	FI Cola	0.22	-0.04	0.25
	CC[2] Cola	-0.47	0.12	-0.55*
	FI 1% Chocolate Milk	-0.17	0.17	-0.25
	CC[2] 1% Chocolate Milk	-0.06	-0.13	-0.01
OW/OB	FI Control	0.06	-0.33	0.32
	FI Fruit Drink	0.37	0.14	0.35
	CC[2] Fruit Drink	-0.38	-0.46	-0.12
	FI Cola	0.53*	0.17	0.51*
	CC[2] Cola	-0.59*	-0.47	-0.37
	FI 1% Chocolate Milk	0.30	-0.13	-0.13
	CC[2] 1% Chocolate Milk	-0.43	-0.22	-0.35
All Subjects	FI Control	-0.04	-0.17	0.07
	FI Fruit Drink	0.27	0.16	0.23
	CC[2] Fruit Drink	-0.36*	-0.35	-0.21
	FI Cola	0.46**	0.18	0.45**
	CC[2] Cola	-0.56**	-0.36*	-0.47**
	FI 1% Chocolate Milk	0.19	0.02	0.23
	CC[2] 1% Chocolate Milk	-0.34	-0.25	-0.25

Pearson correlation coefficients; n=16 NW, n=16 OW/OB, n=32 all subjects. Abbreviations: BW (kg), body weight; CC, caloric compensation; FFM, fat-free mass (kg); FI, food intake (kcal); FM, fat-mass (kg); NW, normal weight; OW, overweight; OB, obese. *P < 0.05, **P < 0.01. [1] FM and FFM estimated from the sum of skinfold measurements at four points (159). [2] CC (%) = [control intake (kcal) – treatment intake (kcal)/ kcal in preload treatment] x 100.

5.3.9. Disinhibition, Dietary Restraint and Emotional Eating

Average DEBQ scores did not differ significantly between NW and OW/OB boys (P=0.24) including restraint (P=0.24), overall disinhibition (P=0.12), specific emotional disinhibition (P=0.35) or external disinhibition (P=0.26). However, OW/OB boys had higher scores for diffuse emotional disinhibition than NW boys (P=0.02) (6.6 ± 0.61 versus 4.6 ± 0.53). DEBQ scores were not consistently associated with FI or CC in boys despite two positive associations in NW boys between restraint and FI after 1% chocolate milk (r=0.56, P=0.02) and diffuse emotional disinhibition and FI after the fruit drink (r=0.51, P=0.04).

5.3.10. Treatment Dose (kcal/kg BW)

In OW/OB, treatment dose (kcal/kg BW) was inversely associated with FI after cola ($r=-0.51$, $P=0.04$). There were no other significant associations between FI and treatment dose observed in either group (**Table 5.11.)**

Table 5.11. Associations between treatment dose (kcal/kg BW) and food intake

	Treatment	Treatment Dose (kcal/kg BW)
NW	Fruit Drink	-0.17
	Cola	-0.26
	1% Chocolate Milk	0.11
OW/OB	Fruit Drink	-0.40
	Cola	-0.51*
	1% Chocolate Milk	-0.28

Pearson correlation coefficients; NW; n=16, OW/OB; n=16. Abbreviations: NW, normal weight; OW/OB, overweight/obese. *P < 0.05.

Chapter 6. General Discussion

6.1. Discussion

The results of this study support the hypothesis that 1% chocolate milk increases meal-time satiation and decreases short-term FI to a greater extent than other isovolumetric SSB in NW and OW/OB boys, but the effect was not diminished in OW/OB boys. Boys reduced FI significantly after cola and 1% chocolate milk compared to the water control. Boys also reduced FI significantly after 1% chocolate milk compared to the fruit drink and cumulative FI was highest after the fruit drink compared to the water control.

1% chocolate milk was expected to suppress FI to a greater extent than the other caloric treatments for several reasons. First, the 1% chocolate milk treatment contained approximately 13 g of protein. Protein is known to suppress FI more than carbohydrate or fat in adults [91-96]. Of the total milk protein content, 20%, or 2.6 g was whey protein which has been observed to suppress FI in children [12]. In a recent study comparing the effect of glucose and whey protein on short-term FI, a 50 g whey protein preload suppressed FI in NW, but not OB 9-14 year old boys. However, the effect of whey protein on CC was lower 30 min later in OB (39%), compared to NW (91%) boys ($P<0.05$) [12]. Similar to the results of this study in which CC after 1% chocolate milk was lower in OW/OB (61%), compared to NW boys (90%), although the effect was not statistically significant. Power analysis indicates that a sample size of ~48 boys would be required to detect a difference in CC between NW and OW/OB boys after the 1% chocolate milk treatment with a power of 0.80.

The other major milk protein, casein, accounts for approximately 80% of milk's protein content [125, 126] and encompassed the remaining 10.4 g of protein in the 1% chocolate milk treatment. Whey protein has a stronger effect on satiety when compared to casein [99]. Adults consumed significantly less energy at a test meal 90 min after a 48 g whey protein preload, compared to after an isoenergetic casein preload [99]. Variations in FI after the consumption of whey protein and casein, may be related to differences in their digestive and absorptive properties [121]. Whey protein and casein are labeled as 'fast' and 'slow' proteins respectively [99]. During digestion, casein coagulates in the stomach due to the precipitation of gastric acid [126]. This slows the rate of digestion [61] and gastric emptying resulting in a smaller postprandial rise in plasma amino acids. Whey protein however, is digested more rapidly [61] which increases the availability of plasma amino acids [121] and promotes satiety [99]. Whey

protein also induces the release of several gut peptides including CCK [123], GLP-1 [66] and PYY from the intestinal cells [124], which contribute to satiety.

Despite a significant reduction in FI after 1% chocolate milk, boys also reduced FI significantly after cola, but not after the fruit drink. This was not expected because of the similar energy content of cola and the fruit drink which contained 158 kcal and 154 kcal respectively. However, the glucose:fructose ratios of the treatments differed at 0.7 g and 1.1 g for cola and the fruit drink, respectively. The greater amount of fructose in cola compared to the fruit drink was perhaps the major determinant of FI. This is consistent with the finding that fructose increases satiety and decreases FI to a greater extent than glucose [86]. In adults, a 50 g fructose preload reduced FI at a test meal provided 2 h later by 500 kcal and 200 kcal more than a 50 g glucose preload and a water control, respectively [85]. The ability of fructose to suppress FI is attributed to its absorption rate within the small intestine. The absorption of fructose is slower than glucose [87], and is hypothesized to contribute to satiety due to increased contact time with GI receptors with initiate satiety signals [88]. This indicates that the composition of the treatments, rather than their energy content affected FI.

The significant suppression of FI after cola and 1% chocolate milk may have also been related to the similar sugars composition of the treatments. In comparison to the 1% chocolate milk treatment which contained sucrose, the principle sweetener in the cola treatment was HFCS which is available in two forms; HFCS-42 and HFCS-55 [234]. HFCS-55 is a liquid mixture composed of 55% fructose and 45% glucose which is commonly used in soft drinks [234]. When HFCS is contained in a liquid solution such as cola, the glucose and fructose molecules exist freely as individual monosaccharides [235]. Conversely, sucrose is a disaccharide composed of equal amounts of fructose and glucose molecules which are covalently bonded [236]. During digestion, the covalent bond between the glucose and fructose molecules in sucrose are broken down in the brush-border cells of the small intestine by sucrase, to produce free glucose and free fructose molecules [236]. Therefore, the glucose and fructose molecules within the cola and 1% chocolate milk treatments would be absorbed in their free-form within both beverages [237] which would lead to an increase in satiety and reduction in subsequent FI [77]. This is consistent with the significant suppression of FI after the cola and 1% chocolate milk treatments.

Although CC scores did not differ significantly between the groups, NW boys compensated almost perfectly for cola (96%) and 1% chocolate milk (90%). OW/OB also compensated to a lesser extent for calories in the cola (40%) and 1% chocolate milk (61%) treatments. Treatments were given in equivolumetric 350 ml doses to provide an amount similar to volumes readily available. When the treatment dose was expressed relative to the BW of subjects in kg, OW/OB boys received fewer calories per kg BW of the 1% chocolate milk treatment compared to NW boys (5.43 kcal/kg BW vs. 4.13 kcal/kg BW). Furthermore, the treatment dose of cola was inversely associated with FI in OW/OB but not NW boys, suggesting that the fixed treatment dose diminished the effect on FI in OW/OB boys. Overall, OW/OB boys had lower CC scores than NW boys indicating that the physiological mechanisms controlling FI in the OW/OB group may have been compromised. A power analysis revealed that a sample size of 186, 52 and 48 subjects would be needed to detect a significant difference in CC between NW and OW/OB boys after the fruit drink, cola and 1% chocolate milk treatments, respectively.

Body composition was measured in this study because in previous research, there was an association with FI in NW, but not OW/OB 9-14 year old boys after caloric beverages [12]. Results from the current study showed that BW was positively associated with FI and inversely associated with CC in OW/OB but not NW boys. This suggests that in the OW/OB group, larger boys consumed more at the test meal and had lower compensation for the calories in the cola treatment. OW/OB boys had a CC score of 40% after the cola treatment compared to NW boys who had a score of 96%. Differences in body composition may affect the physiological regulation of short-term FI in boys. Increased FM has been shown to impede the release of, as well as the sensitivity to satiety hormones in adults including ghrelin, leptin and insulin [12] which function in FI regulation. Despite the associations among body composition, FI and CC, the sugar-sweetened drinks suppressed FI similarly in NW and OW/OB boys indicating that the effect of the drinks was not diminished in the OW/OB group.

Emotional disinhibition, external disinhibition and dietary restraint are eating behaviors hypothesized to affect FI [231]. The DEBQ was used in this study to assess the presence of these behaviors. High scores on the DEBQ have been linked to an increased risk of being OW in adolescents [238]. Average DEBQ scores including restraint, overall disinhibition, specific emotional disinhibition and external disinhibition did not differ significantly between the groups,

although OW/OB boys had higher scores for diffuse emotional disinhibition than NW boys. This indicates that OW/OB boys were perhaps more likely to eat when bored. DEBQ scores were not consistently associated with FI or CC, despite two positive associations between restraint and FI after 1% chocolate milk, and diffuse emotional disinhibition and FI after the fruit drink in NW boys. Therefore, results from the DEBQ do not provide an explanation for the effect of the treatments on FI.

The suppression of FI after cola and 1% chocolate milk also decreased subjective appetite in boys. When subjective appetite scores were corrected for the energy content of the treatment, fullness was higher after cola, and PFC was lower after 1% chocolate milk compared to the fruit drink. This indicates that on a kcal basis, cola and 1% chocolate milk were strong regulators of subjective appetite. Despite these subjective appetite ratings, OW/OB boys consumed more at the test meal after all of the treatments compared to NW boys, indicating that OW/OB boys may be less able to associate feelings of hunger with FI. When subjective appetite scores were correlated with FI at the test meal, PFC was found to be the strongest predictor of FI as indicated by consistent associations between PFC scores and FI. Conversely, DTE was the weakest predictor of FI. Overall, the associations between subjective appetite and FI were more variable in NW boys, indicating that there are differences in the ability of NW and OW/OB boys to express and perceive subjective appetite sensations.

Subjective sweetness of the treatments was measured in this study because sweetness has been hypothesized to stimulate appetite and FI in adults [239, 240]. Subjective sweetness ratings differed among treatments and the fruit drink was rated as significantly sweeter than cola and 1% chocolate milk. However, subjective sweetness was not consistently associated with FI, except between cola and FI in NW boys. Similarly, the mean sweetness ratings were not associated with the mean FI for the three sugars-sweetened treatments, therefore sweetness was not a factor affecting FI. Similarly, 1% chocolate milk was rated as more pleasant than cola, although pleasantness was not associated with FI after any of the treatments. This is consistent with previous research which found no association between preload pleasantness and FI [139], and suggests that the macronutrient composition, rather than the sweetness or pleasantness of the treatments was likely responsible for differences in FI.

6.2. Methods and Limitations

Preload treatments were provided to all subjects in a 350 ml dose regardless of BW. Providing treatments in an equivolumetric dose may not have allowed for an accurate comparison across groups. NW boys received larger treatment doses when expressed per kg BW compared to OW/OB boys, which may have affected subsequent FI and CC at the test meal. Furthermore, OW/OB boys received a significantly smaller treatment dose of the 1% chocolate milk treatment compared to NW boys. The treatments were given in equivolumetric amounts however, as this would provide subjects with a similar volume to what would be consumed in a standard commercially sugars-sweetened drink. The results provide evidence that the exclusion of chocolate milk from the diet as a means to prevent weight gain is unnecessary. Consuming chocolate milk may help reduce short-term FI in children while also serving as a nutrient-dense beverage.

6.3. Conclusion

In conclusion, cola and 1% chocolate milk suppressed FI in boys, however, the effect on FI was dependent on macronutrient composition, treatment dose and body composition.

Chapter 7. Practical Implications & Future Directions

7.1. Practical Implications

The findings of this study have many practical implications for Dietitians, health professionals, policy makers and parents. In particular, it is imperative to understand that sugars-sweetened drinks are not the cause of obesity and that in fact, several of these beverages have beneficial effects in terms of their ability to suppress short-term FI. The findings of this study also provide a basis for the development of school food and nutrition policies as well as specific dietary guidelines for the pediatric population. Recently, several school nutrition policies have opted to replace the sale of flavored milk and soda with beverages such as water, fruit drinks and fruit juices. However, there are specific components in cola and chocolate milk that enhance satiety including the types as well as the amounts of sugars and proteins. Policy makers must therefore re-evaluate the specific policies pertaining to the types of beverages sold in schools to include flavored milks which are satiating, flavorful and nutrient dense. Furthermore, in terms of dietary guidelines for parents and Dietitians, excluding carbonated cola or flavored milk from the diets of children and youth as a means to prevent weight gain is not supported by the previous research.

7.2. Future Directions

7.2.1. Milk Composition, Satiety and Short-Term Food Intake

Cow's milk contains protein (whey protein and casein), lactose and fat which may perhaps play a role in the regulation of FI. In this study, 1% chocolate milk led to a significant reduction in short-term FI compared to a water control in 9-14 year old boys. Future research should investigate the specific components in cow's milk that enhance satiety. This may involve examining the affect of different types of milk (i.e. skim, 1%, 2%, homogenized, chocolate milk) on short-term FI regulation. This information would allow researchers and health professionals to develop specific dietary guidelines regarding the types of milk that are superior in terms of their ability to reduce appetite and FI in the pediatric population.

7.2.2. Treatment Dose

Treatments were provided to boys in an equivolumetric 350 ml dose. This fixed volume dose provided NW boys with larger treatment doses when expressed per kg BW, compared to OW/OB boys which may have affected subsequent FI at the test meal. Furthermore, treatment

dose was inversely associated with FI after cola in OW/OB boys. This indicates that the treatment dose was perhaps below the threshold required to significantly decrease FI in the OW/OB boys. Future research should provide subjects with a treatment dose based on their BW (kg), to allow for an accurate comparison across groups.

7.2.3. Gastrointestinal Hormones and Short Term FI Regulation

In this study, 1% chocolate milk and cola led to a significant reduction in FI although it is unknown which satiety hormones were responsible for this suppression, and whether differences exist between the amounts of protein-induced, versus carbohydrate-induced satiety hormones in NW compared to OW/OB boys. Currently, research investigating the response of satiety hormones to protein and carbohydrate in adolescent boys is limited; therefore future studies should investigate whether differences in the amounts as well as release of satiety hormones including ghrelin, CCK, GLP-1, PYY and leptin exist between NW and OW/OB individuals. This would provide a better understanding of the physiological mechanisms involved in appetite control in children.

Chapter 8. References

8.1. References

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Chapter 9. Appendices

Appendix 9.1 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 Lorianne Bennett at 902-457-6378.

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Appendix 9.2. Recruitment Letter for Parents

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

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 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 to participate, or to get further information beyond that provided in this letter, please contact Dr. Nick Bellissimo, Principal Investigator or Ms. Kelly Poirier, Project Coordinator 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. Kelly Poirier, Department of Applied Human Nutrition, Mount Saint Vincent University.

Appendix 9.3. Telephone Screening Questionnaire

Department of Applied Human Nutrition

MOUNT SAINT VINCENT UNIVERSITY

Food intake control in children. **Experiment:**

Name: _____

Age:		_____	years	DOB (d/m/y)	_____	Term ____?	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	**cereal**	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? yes/no

Milk, nuts, wheat

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 9.4. 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

Investigators:

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Project Coordinator
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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 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. This experiment is being conducted through the Department of Applied Human Nutrition at Mount Saint Vincent University by Dr. Nick Bellissimo and Ms. Kelly Poirier. 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. Kelly Poirier. 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-ileal, 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 well at all" to "Very well") . 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 remain 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. Kelly 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 x 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'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 9.5. Children's Assent Form

Department of Applied Human Nutrition

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

Children's Assent

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 9.6. Information Session Study Sheet

Subject Information

Name: _____ Phone #: _____

Address:_____

Age: _____ decimal years DOB (d/m/y): _____

Health Status

Weight: _____kg Height: _____cm IBW: _____kg

Skinfold Thickness

Biceps: _____mm Triceps: _____mm Skinfold Sum: _____mm

Supra-ilial: _____mm Subscapular: _____mm Fat Mass: _____%

Include in study?

(Y/N) _____ If yes, subject #: _____ Subject code: _____

If not, why? _____

Forms Completed?

Food acceptability list completed? (Y/N) _____

Consent/assent forms signed? (Y/N) _____

Dutch Eating Behaviour Questionnaire? (Y/N) _____

Standardized breakfast given for next session? (Y/N) _____

Beverage

At all study sessions, your child will receive a sweet beverage. Please indicate whether your child will be able to drink a sweet beverage provided at each session. (Y/N) _____

Lunch

The child will be provided with a pizza meal the day of the study. To enable us to provide your child with a meal that they will enjoy, which type of pizza should we serve? Pepperoni/Cheese?

Name of parent/guardian: _____

Investigator: _____

Date (d/m/y): _____

9.1. Appendix 9.7. 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 _____

Appendix 9.8. Study Day Questionnaires

VAS – Motivation to eat

VAS – Pleasantness of preload

VAS – Pleasantness of test meal

VAS – Sweetness of preload

VAS – Physical comfort

Appendix 9.8a. VAS Motivation to Eat

Time =

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 WEAK _____ Very STRONG

2. How hungry do you feel?

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

3. How full do you feel?

NOT Full at all _____ VERY Full

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

NOTHING at all _____ A LARGE amount

5. How thirsty do you feel?

NOT thirsty at all _____ As thirsty as I have ever felt

Appendix 9.8b. 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 at all pleasant _____ Very pleasant

Appendix 9.8c. 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 at all pleasant _____ Very pleasant

Appendix 9.8d. VAS Sweetness of Preload

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 sweet at all _____ Extremely sweet

Appendix 9.8e. VAS Physical Comfort

Time =

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?

NOT well at all _____ VERY Well

Appendix 9.9. 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?
Never Seldom Sometimes Often Very often

13. Do you have the desire to eat when you are depressed or discouraged?
Never Seldom Sometimes Often Very often

14. Do you have a desire to eat when you are feeling lonely?
Never Seldom Sometimes Often Very often

15. Do you have a desire to eat when somebody lets you down?
Never Seldom Sometimes Often Very often

16. Do you have a desire to eat when you are angry?
Never Seldom Sometimes Often Very often

17. Do you have a desire to eat when you are expecting something unpleasant to happen?
Never Seldom Sometimes Often Very often

18. Do you get the desire to eat when you are anxious, worried or tense?
Never Seldom Sometimes Often Very often

19. Do you have a desire to eat when things are going against you or when things have gone wrong?
Never Seldom Sometimes Often Very often

20. Do you have a desire to eat when you are frightened?
Never Seldom Sometimes Often Very often

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

22. Do you have a desire to eat when you are bored or restless?
Never Seldom Sometimes Often Very often

23. Do you have a desire to eat when you are emotionally upset?
Never Seldom Sometimes Often Very often

24. If food tastes good to you, do you eat more than usual?
Never Seldom Sometimes Often Very often

25. If food smells and looks good to you, do you eat more than usual?
Never Seldom Sometimes Often Very often

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

Appendix 9.10. Test Meal Food Intake Measurement Form

Subject :_____ Pizza Preference:_____

Date: _____

	Treatment	Before	After
Tray 1	Pepperoni(g)		
	Cheese(g)		
Tray 2	Pepperoni(g)		
	Cheese(g)		
Tray 3	Pepperoni(g)		
	Cheese(g)		
Tray 4	Pepperoni(g)		
	Cheese(g)		
	Water(g)		
	Water(g)		

Session: _____

Investigator: _____

Date: _____

	Treatment	Before	After
Tray 1	Pepperoni(g)		
	Cheese(g)		
Tray 2	Pepperoni(g)		
	Cheese(g)		
Tray 3	Pepperoni(g)		
	Cheese(g)		
Tray 4	Pepperoni(g)		
	Cheese(g)		
	Water(g)		
	Water(g)		

Session: _____

Investigator: _____