PHYSIOLOGICAL REGULATION OF SHORT-TERM FOOD INTAKE IN CHILDREN DURING PUBERTY

by

Barkha P. Patel

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Nutritional Sciences
University of Toronto

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Abstract

Three studies were designed to investigate the hypothesis that physiological and environmental variables are both independent and interactive in determining food intake (FI) in children and adolescents during puberty (8 – 18 y old). Study 1 investigated the effect of obesity, sex and pubertal status on appetite hormones in response to a mixed glucose and whey protein (WP) drink in adolescents. Obese adolescents had higher insulin, PYY and lower ghrelin than normal weight (NW) controls, with a more pronounced effect in males. Puberty did not affect insulin, but the change in PYY in response to the drink was greater and ghrelin was lower in mid-late pubertal than pre-early pubertal obese males. To further describe the role of puberty, Study 2 examined the effect of pubertal status on FI following consumption of glucose and WP drinks in male and female children. In mid-late pubertal children, mealtime compensation for energy from glucose was less at 60 than at 30 min, but not for whey. However, compensation for either drink was not different at 30 and 60 min meals in pre-early pubertal children. Finally to demonstrate the interaction between puberty and environmental influences on FI, Study 3 examined the effect of distraction (television viewing, TVV) while eating and pubertal status on food intake after a pre-meal glucose drink in girls. In Study 3, TVV had no effect on FI, however, glucose suppressed FI more with no TVV compared with TVV (24% vs. 10%). In postpubertal girls, glucose reduced FI by ~27% in both the no TVV and TVV conditions, but in peripubertal girls, reduction in FI was 22% without TVV and only 1% while TVV. Thus, the results of this research support the hypothesis that physiological and environmental variables are both independent and interactive in determining FI in children and adolescents during puberty.
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List of Abbreviations

AgRP  agouti-related peptide
ANOVA analysis of variance
ARC  arcuate nucleus
AUC  area under the curve
BMI  body mass index
CART  cocaine and amphetamine-regulated transcript
CCK  cholecystokinin
CDC  Centre for Disease Control
CNS  central nervous system
DPP-IV  dipeptidyl peptidase IV
FI  food intake
FSH  follicle stimulating hormone
GI  gastrointestinal
GIP  glucose-dependent insulinotropic peptide
GLP-1  glucagon-like peptide-1
GnRH  gonadotropin-releasing hormone
HPG  hypothalamic–pituitary–gonadal
IGF-1  Insulin-like growth factor 1
Kcal  kilocalories
LH  luteinizing hormone
LHA  lateral hypothalamic area
NPY  neuropeptide Y
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>NTS</td>
<td>nucleus of the tractus solitarius</td>
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<tr>
<td>NW</td>
<td>normal weight</td>
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<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
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<tr>
<td>PFC</td>
<td>prospective food consumption</td>
</tr>
<tr>
<td>POMC</td>
<td>proopiomelanocortin</td>
</tr>
<tr>
<td>PYY</td>
<td>peptide tyrosine tyrosine (3-36)</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>TVV</td>
<td>television viewing</td>
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<tr>
<td>VMH</td>
<td>ventromedial hypothalamus</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
<tr>
<td>WBISI</td>
<td>whole body insulin sensitivity index</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WP</td>
<td>whey protein</td>
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<td>VAS</td>
<td>Visual Analogue Scale</td>
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INTRODUCTION

Over the last 25 years, obesity has become the most predominant nutritional issue in the world, affecting both adults and children (1). Research on the causative factors of obesity in children has predominantly focused on how the environment affects intake control, whereas the physiological aspects of food intake (FI) remain understudied, especially as it relates to developmental stages of puberty. Thus, it is unclear if disruptions in energy balance occur because the physiological control of FI is diminished in susceptible children or if it is present, but overridden by the environment (2). Examining FI regulation in children is important as overweight/obese children are at high risk of becoming obese adults and developing chronic diseases associated with increased adiposity.

The overall objective of this research is to develop an understanding of physiologic and environmental variables and how they interact to affect appetite and short-term FI regulation in children during puberty. The specific objective was to examine the interactions among body weight, pubertal status and sex with the mealtime environment on FI regulation in response to glucose and whey protein (WP) beverages in 9 – 18 y old children and adolescents.

Three studies were conducted. Study 1 examined the effect of obesity, sex and pubertal status on subjective appetite and appetite hormones in response to a mixed glucose and WP drink in children and adolescents. To further describe the role of puberty, Study 2 examined the effect of pubertal stage on subjective appetite and FI after pre-meal glucose and WP drinks in children. Finally to demonstrate the interaction between puberty and environmental influences on FI, Study 3 examined the effect of pubertal stage and television viewing (TVV) on subjective appetite and FI after a pre-meal glucose drink in girls.

Understanding the physiological and environmental factors and the interactions among them, which contribute to energy imbalance during a critical stage of development of FI control
in children, will provide a foundation for advice aimed at preventing and managing excess energy intake.
2 LITERATURE REVIEW

2.1 Introduction

To provide a background for the research conducted, this literature review comprises five sections. The first section provides an introduction to the prevalence, consequence and causes of childhood obesity. In the second section, the physiology of food and appetite regulation in children and adolescents is reviewed, while the third section examines the role of puberty and the hormonal regulation of FI during this period. Section four provides a review of environmental factors with a specific focus on the relationship between TVV and appetite regulation in children and adolescents. The final section reviews the effect of macronutrient composition on FI regulation in children.

2.2 Overweight and Obesity in Children and Adolescents

The prevalence and severity of obesity has increased steadily over the past three decades. From a global perspective, obesity is a serious public health issue affecting adults and children in both the developed and developing world. In 2006, direct costs of overweight and obesity in Canada were estimated at 6 billion dollars (3.6 billion for obesity alone) (3). According to the 2004 Canadian Community Health Survey, the prevalence of obesity has almost tripled in adolescents aged 12 – 17 over the past 25 y (4). More recent data from the Canadian Health Measures Survey (2007-2009) revealed that the prevalence of childhood overweight and obesity has remained relatively steady between 2004 and 2007-09 in 6 - 17 year olds (Cost of Obesity in Alberta for 2005), with ~17% overweight and ~9% obese (5). Despite this apparent plateau,
prevention of childhood obesity is crucial because obese children are highly susceptible to obesity-related diseases, such as diabetes, and are at increased risk of becoming obese adults.

2.2.1 Diagnosis

Diagnosis of overweight and obesity in children and adolescents (2 – 17 y) is based on their BMI, which is calculated by dividing weight in kilograms by height in metres squared (1). Since children are in a state of growth, BMI cut-offs vary with the age and sex of the child. Currently, there are three sets of criteria that are most commonly used to distinguish overweight and obesity in children: i) The International Obesity Task Force criteria (6), iii) the Centre for Disease Control (CDC) sex-specific BMI-for-age growth charts (7) and iii) the World Health Organization (WHO) growth charts (8).

In Canada, the WHO growth charts (8) are the recommended reference for the assessment of growth of Canadian children by Dietitians of Canada, Canadian Paediatric Society, The College of Family Physicians of Canada and Community Health Nurses of Canada. The reconstructed WHO Reference 2007 charts were developed for school-aged children and adolescents (5 – 19 y) and address the limitations of the 1977 American National Centre for Health Statistics growth charts. The 2007 charts utilized the WHO Child Growth Standards curves for children under five years old, which represents the prescribed gold standard for childhood growth. According to the WHO Reference 2007 charts, overweight is defined as a BMI between the 85th and 97th percentile and obesity is greater than the 97th percentile, which correspond with the adult cut-offs for overweight and obesity (9).
2.2.2 Health Consequences of Obesity

Children are now frequently affected with adverse health conditions once thought only to affect adults. Furthermore, puberty is a time of increased susceptibility to the development of obesity-related health issues as a result of the dramatic changes in physiology, body composition and energy demands associated with this process (10). Excess adiposity in children can lead to cardiovascular complications including hypertension and atherosclerosis and increased prevalence of metabolic disorders such as insulin resistance, metabolic syndrome, dyslipidemia and type 2 diabetes (10). Asthma and obstructive sleep apnea, both pulmonary disorders, are estimated to affect 7-9% and 1-5%, respectively of the pediatric population (10). Obstructive sleep apnea is characterized by snoring and partial (hypopneas) or complete (apneas) obstruction of the upper airway; it is associated with intermittent oxyhemoglobin desaturation, sleep disruption, and fragmentation (10). The prevalence of this disorder amongst obese children and adolescents can be as high as 60% (11). Furthermore, depression and low self-esteem encompass the broad range of psychosocial issues that impact obese children and that can occur alongside the cardiovascular, metabolic and pulmonary complications. The cumulative effects of these obesity-associated diseases can reduce the lifespan, as well as the quality of life, of those affected.

2.2.3 Etiology of Obesity

Obesity arises when energy intake exceeds energy expenditure; however, the causes behind increases in energy intake are unclear. Rising obesity rates in children are due to a complex interplay between genetic, environmental and behavioural factors (12). However, our
genetic make-up has not drastically changed concurrent with the rise in obesity (13), suggesting that increases in weight are primarily due to environmental and behavioural factors affecting energy balance. These factors include an inexpensive food supply, ready availability of added sugars in foods/beverages (14), consumption of high energy-dense foods, food variety, increased portion sizes (2, 15) and more time spent in sedentary pursuits (ie. TVV) (16).

Changes in the environment have also contributed to childhood obesity: the structure of the built environment, schools/day care and parenting styles (13). However, physiologic factors affecting intake control during childhood and adolescence, including age, sex, pubertal stage, body fatness and the macronutrient composition of food have received little consideration. It is unclear whether obesity develops in susceptible individuals because physiological mechanisms of FI control are initially compromised or if they are overridden by factors in the environment and become compromised. Understanding how these determinants interact to disrupt the delicate balance between energy intake and energy expenditure is necessary to prevent overweight and obesity in children.

2.3 Food Intake Regulation in Children and Adolescents

Research on the regulation of FI has intensified with the increased prevalence of overweight and obesity in all age groups. The majority of work has focused on adults and searches for effective dietary and drug treatments. However, little attention has been given to understanding how FI and body weight is regulated in children of healthy body weights, in obese children, or during development of puberty and how this regulation is affected by environmental factors. The following provides a review of the limited literature on these factors.
2.4 Physiology of Food Intake Regulation in Children and Adolescents

2.4.1 Overview of food intake regulation

Food intake regulation is a precise biological process that involves the integration of complex homeostatic mechanisms from the central nervous system (CNS) with peripherally derived signals of the body’s nutritional and energy status. Input is received from sensory properties of food, mechanical and chemical receptors in the gastrointestinal (GI) tract, circulating metabolites, and gut hormones, which are involved in the short-term regulation of FI (17, 18). Long-term regulation of FI is mediated by adiposity signals leptin and insulin. The control centers for FI regulatory signals and energy expenditure are contained within the limbic system of the brain (19). These signals are integrated through an intricate neuronal circuitry involving the ventromedial hypothalamus (VMH) and the nucleus of the tractus solitarius (NTS), which translates them into information regulating meal size and duration, time to the next meal, the amount of food consumed over the short-term or long-term, and possibly the composition of food and total energy intake (Fig. 2.1). The VMH is composed of the ventromedial nucleus and arcuate nucleus (ARC) and mediates afferent and efferent neuroendocrine signals. VMH neurons contain receptors for and receive afferent signals related to adiposity (leptin), nutrient metabolism (insulin), hunger (ghrelin), and satiety (peptide tyrosine tyrosine, PYY) (20). The ARC contains two distinct neuronal populations with opposing effects on FI: first, neurons which coexpress orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP); second, neurons which coexpress anorexigenic proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) (21).
The VMH transmits these afferent signals via the paraventricular nucleus and lateral hypothalamic area through neurons containing the melanocortin-4 receptor; appetite is either stimulated or suppressed (22). The autonomic nervous system is then activated by efferent signals; sympathetic activation promotes energy expenditure via gluconeogenesis and lipolysis whereas parasympathetic activation promotes energy storage through lipogenesis (19). Hunger and satiety neuronal circuits in the VMH interact with other regions of the limbic system (23), including the ventral tegmental area (VTA) and nucleus accumbens; these limbic structures of the hedonic pathway make FI rewarding. Neurotransmission of dopamine from the VTA to the nucleus accumbens mediates the reward value of food (24). This neurotransmission is suppressed by leptin and insulin signaling adipose and nutrient sufficiency in normal circumstances (25). However, consumption of highly palatable, sweet and high-fat food can weaken satiety signals and motivate energy intake independent of energy need (49).
**Figure 2.1:** Regulation of long term and short term FI. Leptin and insulin regulate FI via binding activation of POMC/CART and inhibition of NPY/AgRP receptors within the ARC, leading to downstream signalling at nuclei within the PVN and LHA, thereby decreasing FI. The NTS is responsible for integration of both the central as well as GI signals regulating FI.

PVN, paraventricular nucleus; LHA, lateral hypothalamic area; ARC, arcuate nucleus; NPY, neuropeptide Y; POMC, proopiomelanocortin; AgRP, Agouti-related peptide; CART, cocaine and amphetamine regulated transcript; PYY, peptide YY; GLP-1, glucagon-like peptide 1; CCK, cholecystokinin; GI, gastrointestinal; NTS, nucleus of the solitary tract; FI, food intake
2.4.2 Short-term Food Intake Regulation

Many of the short-term signals that regulate FI are activated by GI responses to food ingestion. The GI tract is the largest endocrine organ in the body (26) and contains receptors that respond to the physiochemical properties of food and recognize the macronutrient composition of the food ingested.

Prior to a meal, ghrelin, which is secreted primarily by the gastric fundus and proximal small intestine, is present at peak levels within the body (27-29). Ghrelin stimulates FI, and is the only gut hormone known to do so. Ghrelin is an energy sensor and postprandial suppression of ghrelin is proportional to the calories consumed (30, 31). Post-prandial suppression is mediated by increased insulin levels (32, 33). Ghrelin reaches trough levels within 60 minutes of meal consumption (34).

The ingestion of food results in the presence of macronutrient breakdown products in the GI tract. The passage of these pre-absorptive products gives rise to a number of signals that are transmitted to the brain through direct or indirect stimulation of vagal receptors (35). Furthermore, the products of digestion (post-absorptive signals) enter the circulation to provide direct signals to the brain via their effect on neurotransmitter systems known to be involved in regulating feeding behavior (36).

For example, the presence of food in the upper GI tract stimulates the release of the satiety hormone PYY. PYY is produced mainly by L cells in the ileum and colon and is released in proportion to caloric load (31, 37). PYY release is first stimulated by atropine-sensitive neural projections in the foregut, then directly by the hindgut in response to nutrient stimulation (38). PYY levels are low during fasting, but rise rapidly to peak levels within 60 minutes of a meal,
falling back to baseline after approximately 6 hours, depending on the composition of the food ingested (39, 40).

Short-term FI is also regulated through the release of more than 50 gut hormones (41) involved in the processing of the nutrients derived from digestion and absorption, including, glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), amylin and pancreatic polypeptide (42), all of which induce satiety.

In the obese adult, several of these gut hormone responses are altered. For example, PYY levels are found to be lower at fasting and following a meal in obese compared to lean individuals. Ghrelin levels are also found to be negatively correlated with body weight, with lower ghrelin in obese compared to NW individuals (43-47).

Although many gut hormone genes are expressed, the appetite-related effects of relatively few have been identified with respect to FI regulation, particularly in the pediatric age group. These include PYY and ghrelin, the most studied gut hormones in children and adolescents. Leptin and insulin can also act synergistically with PYY and ghrelin to regulate short-term FI (36). Details of the most studied hormone actions will be outlined in the following sections.

2.4.3 Long-term Food Intake Regulation

Signals arising from adipose tissue (leptin) and the pancreas (insulin) exert their actions by binding to receptors in the hypothalamus and hindbrain (48). These hormones give an indication of longer-term energy stores and affect the amount of food consumed over several days, weeks and months. In NW individuals, leptin is involved in long-term energy regulation by inducing central appetite inhibition and stimulating energy expenditure by increasing central
sympathetic tone (49, 50). Leptin levels circulate in proportion to the amount of adipose tissue in both NW and overweight/obese individuals. However, long-term energy excess resulting in obesity may also alter set-points regulating energy balance, leading to a conservation of body fat. Thus, in spite of increased levels of leptin in overweight individuals, appetite and FI are not suppressed because increased fat mass also leads to central leptin resistance at the level of the hypothalamus (51, 52). Peripheral and central leptin resistance may then lead to impaired appetite suppression. Similar to leptin, insulin levels are significantly higher in obese versus NW children (53, 54), indicating peripheral and central insulin resistance (50). Children with the greatest insulin resistance and highest insulin concentrations have the greatest increases in body weight over time (55), which may be due to increased FI, since insulin resistance is positively correlated with energy intake in overweight children (56). Successful weight loss leads to reduction of hyperinsulinemia and improved insulin sensitivity (57, 58), as well as lower leptin levels (59, 60).

However, alterations in the hormonal mechanisms that control hunger and fullness make it harder to lose weight or keep it off due to these changes. Chronic insulin in obesity inhibits leptin signaling centrally; decreased leptin signaling is interpreted by the VMH as starvation, leading to sympathetic reduction to conserve energy and parasympathetic activation to store energy (61). Furthermore, excess energy consumption over time may induce obesity and subsequent hyperinsulinemia, promoting insulin-mediated deposition of excess energy in the form of adipose tissue (56). Less is known about the role of other hormones such as ghrelin and PYY on body weight regulation in children.

2.4.4 Appetite Regulation in Children and Adolescents
Although many hormones are involved in appetite and FI, few have been examined in children. Only a few studies have examined the incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). The most frequently studied have been ghrelin and PYY in the context of their association with insulin. Leptin has also been studied in longer-term FI studies in children. Thus, the following section will discuss the role of ghrelin, PYY, insulin and leptin on appetite regulation in children and adolescents.

2.4.4.1 Ghrelin Physiology and Mechanism of Action

Ghrelin is the only known orexigenic hormone and is secreted primarily by the oxyntic cells in the gastric fundus (27-29), but also by the duodenum, ileum, caecum and colon (28). Ghrelin circulates in both acylated (active) and deacylated forms, with the former initiating ghrelin’s orexigenic effects (62). Acylation occurs at its serine amino acid at position 3 by n-octanoyl acid (29), and allows for ghrelin to cross the blood-brain barrier and bind to its receptor. Ghrelin’s ability to induce hunger is mediated by activating NPY/AgRP neurons in the ARC of the hypothalamus (63). Ghrelin binds to presynaptic terminals of NPY and POMC neurons, respectively stimulating and inhibiting their activity and peptide release (64). Hormonal signals, such as the post-prandial increase in insulin (65-67) and other satiety hormones (PYY, GLP-1) (40, 68) prevent the release of ghrelin.

2.4.4.2 Ghrelin and Appetite Regulation in Children and Adolescents

Serum ghrelin levels are influenced by short-term and long-term changes in energy homeostasis, including fluctuations in glucose, insulin and somatostatin levels. Ghrelin
stimulates appetite via the afferent vagal nerve (28). Circulating ghrelin increases in the fasting state under sympathetic innervations, and decreases in the postprandial period, potentially regulated by neurally transmitted nonvagal intestinal signals, as well as by an increase in insulin secretion (32, 33, 56), but not gastric distention or vagal neurologic control (69). However, some studies support a role for vagal activity being necessary for ghrelin suppression after meal consumption (70, 71). Circulating ghrelin levels in lean subjects are pulsatile and follow diurnal and ultradian rhythms, with levels rising at night and prior to times of habitual meal intake, i.e. entrained to usual meal intake times in day (72). Ghrelin is inversely associated with BMI and fasting ghrelin concentrations are lower in obese (43-47) compared to NW children. The exact mechanism for lower ghrelin in obesity is not fully understood, but may be a compensatory mechanism to prevent overfeeding and/or a result of metabolic changes associated with obesity, such as insulin resistance (73).

Ghrelin concentrations are positively related to appetite scores and inversely related to the intermeal interval (34, 74, 75). Post-prandial suppression of ghrelin primarily responds to caloric content and is greatest following a larger, high-calorie meal (30). However, macronutrient composition is a factor determining post-meal decreases in ghrelin.

In children, a 75 g glucose load suppressed total ghrelin levels from baseline similarly in lean and obese subjects, indicating equal sensitivity to the preload regardless of overall lower baseline ghrelin levels in the obese children (44). Similarly, another study found that similar percent suppression in ghrelin between NW and overweight prepubertal children occurred alongside significantly higher insulin response in the overweight children (76). As mentioned, hyperinsulinemia in obesity appears to mediate the suppression in ghrelin, supported by positive associations between ghrelin and insulin sensitivity independent of adiposity (76).
However, two studies found that following an oral glucose test (0.75g/kg and 1.75g/kg, maximum 75g), a decrease in active acylated ghrelin levels from baseline in obese children was blunted compared to lean control at 30 and 60 minutes, suggestive of a delayed ghrelin response in the obese (46, 77).

Studies using mixed meals yield conflicting results in children. For instance, total ghrelin in obese adolescent females decreased transiently after a mixed meal (~480 kcal, 55% carbohydrates, 25% protein and 20% fat) (53), and decreased rapidly in overweight boys after a mixed meal (~688 kcal, 36% carbohydrates, 16% protein and 48% fat) at 30 and 60 min (78). However, total ghrelin did not change in overweight children after a standardized breakfast (400 kcal, 60% carbohydrate, 10% protein and 30% fat) and a mixed meal lunch (600 kcal, 60% carbohydrate, 10% protein and 30% fat) (54). The conflicting results suggest that post-prandial ghrelin suppression is dependent on specific macronutrient intakes. In both NW and obese children, a high protein meal (440 kcal, 36% carbohydrate, 44% protein, 20% fat) gradually lowered ghrelin levels, with the absence of a rebound, while a high carbohydrate meal (430 kcal, 88% carbohydrate, 2% protein, 10% fat) rapidly lowered ghrelin levels to a nadir at 60 min, but which subsequently increased over time (45). The authors suggested that while both meals suppressed ghrelin levels, the high protein meal prevented the rebound in ghrelin, consistent with lower subjective ratings of hunger observed in the obese group after the high protein meal (45).

Most recent studies have measured active ghrelin levels, which is the form displaying orexigenic effects, and have produced inconsistent outcomes among themselves and in comparison with reports measuring total ghrelin. Interestingly, active ghrelin was not suppressed after consumption of a high carbohydrate (~440-543 kcal, ~66% carbohydrate, ~16% protein, ~18% fat), high protein (~269-403 kcal, ~16% carbohydrate, ~64% protein, ~20% fat) or high fat
(~400-581 kcal, ~18% carbohydrate, ~17% protein, ~65% fat) breakfast in NW and obese adolescent girls (47). Paradoxically, the high carbohydrate meal increased active ghrelin in obese compared to NW girls, who ingested more calories at the breakfast (47). One recent study demonstrated (79) that a small mixed meal transiently stimulates active ghrelin levels in both lean and obese adolescents during the first 30 min of feeding. Thereafter, active ghrelin decreased to a nadir in lean adolescents, but this was attenuated in obese adolescents, suggesting that lower ghrelin after caloric intake may contribute to higher energy intakes and reduced satiety in obese children (79). Thus, inconsistencies in the data warrant further understanding of the role of active ghrelin in children in response to individual macronutrients and mixed meals.

2.4.4.3 PYY Physiology and Mechanism of Action

PYY is released into circulation from the L cells of the distal gut (37) and exists in two circulating forms: PYY (1–36) and PYY (3–36). PYY (3–36) is the major anorectic signal (80–82) and is produced by cleavage of the N-terminal Tyrosine-Proline residues from PYY (1–36) by the enzyme dipeptidyl-peptidase IV (DPPIV) (83). PYY (3–36) can cross the blood-brain barrier to suppress FI by activating its NPY Y2 receptor at the ARC. Receptor activation leads to the inhibition of NPY/AgRP neurons and the activation of the anorexigenic POMC neurons, resulting in increased expression of POMC within the ARC (84).

2.4.4.4 PYY and Appetite Regulation in Children and Adolescents

In the fasting state, PYY levels are low, rise within 15 min in response to nutrient ingestion, peak at 1h and remain elevated for several hours (85). The amount of PYY released
postprandially is directly proportional to the amount of food consumed. Increased PYY delays gastric emptying and reduces gastric, pancreatic and intestinal secretion and GI mobility (86, 87). The effect of obesity on PYY concentrations is uncertain; overweight/obese children and adolescents (7 – 18 y) have either similar (45, 47, 53, 79), higher (54) or lower (88) fasting PYY concentrations than NW children.

Plasma PYY concentrations are affected by obesity and the composition of the test meal. Following an oral glucose tolerance test (1.75 g/kg, maximum 75g), total PYY levels increased significantly in lean children from baseline until 120 minutes, while obese children showed an increase only at 30 minutes (46). Total PYY increased by ~27% after a mixed meal lunch (600 kcal, 60% carbohydrate, 10% protein and 30% fat) in overweight children (7 – 11 y) (54), but did not change in obese females (12 – 18 y) after consumption of a liquid preload (~480 kcal; 55% carbohydrates, 25% protein and 20% fat) (53).

PYY release from macronutrient specific meals has recently been examined in children. Compared to the ingestion of equicaloric carbohydrate or protein meals, fat promotes the greatest release of PYY in adult studies (85, 89). In contrast, PYY concentrations did not differ after a high-fat (590 kcal, 36% carbohydrate, 12% protein, 52% fat) compared to a moderate-fat (590 kcal, 61% carbohydrate, 12% protein, 27% fat) meal (90) in obese boys (8 – 12 y). Another study compared total PYY responses to high carbohydrate, high protein and high fat meals in NW and obese children (7-11 y). In both groups, the high fat meal (417 kcal, 17% carbohydrate, 2% protein, 81% fat) increased total PYY, with levels declining slowly compared to the high carbohydrate meal (430 kcal, 88% carbohydrate, 2% protein, 10% fat). However, the high protein meal (440 kcal, 36% carbohydrate, 44% protein, 20% fat) steadily increased total PYY levels over time in both groups of children, whereas total PYY peaked at 30 min after the high
carbohydrate meal, with levels declining afterwards (45). Except with the high protein meal, obese children had attenuated PYY responses indicated by lower AUCs compared to NW children (45), consistent with the results of another study in obese children (79). In obese children, total PYY AUC was greater after the high protein meal compared to the other macronutrient specific meals (45).

Furthermore, only one study has specifically examined the active form of PYY in response to macronutrient ingestion by children. In contrast to total PYY increasing after a high fat compared to a high carbohydrate meal in adults (89), PYY (3-36) levels were lower in obese than NW girls after a high fat (~400-581 kcal, ~18% carbohydrate, ~17% protein, ~65% fat) breakfast, but not after a high carbohydrate (~440-543 kcal, ~66% carbohydrate, ~16% protein, ~18% fat) or high protein (~269-403 kcal, ~16% carbohydrate, ~64% protein, ~20% fat) breakfast (47). A lower percentage of fat content in the meals may explain the failure of fat to stimulate PYY (3-36) in adolescents compared to adults, but further studies are needed to clarify this finding. These results indicate the potential of high protein diets to increase satiety in the short-term and lower FI in obese children.

2.4.4.5 Insulin Physiology and Mechanism of Action

Insulin regulates glucose homeostasis by increasing glucose uptake into adipose tissue and muscle, as well as by reducing hepatic glucose production (91). When insulin secretion is dysregulated, blood glucose concentrations rise, and the resultant hyperglycaemia is a hallmark of diabetes mellitus. Insulin enters the CNS by crossing the BBB through receptor-mediated transport (92). In the ARC, insulin stimulates POMC neurons and inhibits NPY/AgRP neurons (48). However, it was shown that insulin’s suppression of glucose production operates through
NPY/AgRP, and not POMC neurons. When insulin receptors in AgRP neurons of mice were knocked out, hepatic glucose production was not suppressed, but this effect was not observed when insulin receptors were knocked out in POMC neurons in response to elevated circulating levels of insulin (93). Thus, insulin operates through NPY/AgRP neurons to reduce hepatic glucose production.

Furthermore, insulin’s central effects on energy intake include decreasing motivation to eat and the pleasurable aspects of food (94). However, central insulin resistance in obesity promotes increased glucose dysregulation and interferes with meal-associated insulin effects on dopamine reuptake. The failure of insulin to control the reward system at the nucleus accumbens leads to increased FI and reduced satiety (94).

2.4.4.6 Insulin and Appetite Regulation in Children and Adolescents

Insulin increases in response to an oral glucose tolerance test (OGTT) in both NW and obese children, but the response is significantly greater in the obese children (44, 46, 95). Compared to NW females, obese adolescent females have a greater insulin response after a mixed meal (~480 kcal; 55% carbohydrates, 25% protein and 20% fat), but similar glucose levels (53).

2.4.4.7 Leptin Physiology and Mechanism of Action

Leptin enters the CNS in proportion to plasma concentration (96). In the ARC, leptin primarily acts through the activation of neurons producing POMC and CART, which inhibit
appetite, and inhibition of those producing NPY and AgRP, which stimulate appetite (97). In addition to its satiety actions in the CNS, leptin interacts synergistically with CCK (98), to enhance the satiating effect of this short-term signal. Being female and having a higher BMI is significantly and independently associated with increased serum leptin levels in overweight children and adolescents (99).

2.4.4.8 Leptin and Appetite Regulation in Children and Adolescents

Leptin concentrations do not change acutely (~3-4 h) in response to meals and most studies do not observe associations between leptin and subjective measures of appetite pre- and post-prandially when individuals are in energy balance (100-102). However, in cases of energy imbalance, leptin is negatively correlated to appetite, such as energy restriction when leptin concentrations are low (103, 104) or an energy surplus when leptin concentrations are high (104). Leptin is important in the long-term regulation of FI particularly when energy balance is distorted.

However, the macronutrient composition of a meal and body weight has an effect on longer-term postprandial leptin concentrations. A high-fat, low-carbohydrate meal (20% carbohydrate, 20% protein, 60% fat) compared to a low-fat, high-carbohydrate meal (60% carbohydrate, 20% protein, 20% fat), results in reduced circulating leptin concentrations over a 24-h period in NW women, suggesting that circulating leptin levels are not only an indication of adiposity or energy balance, but are also affected by the macronutrient content of a meal (105). In severely obese women, a moderately high carbohydrate meal (60% carbohydrate, 20% protein, 20% fat) adjusted based on the kcal needs of the patient led to lower post-prandial leptin
levels over time compared to lean controls (106), suggesting an impairment of post-prandial leptin regulation in obesity.

The satiety effects of leptin have not been the focus of short-term studies in children. However, one study showed that prepubertal obese children given a high carbohydrate meal (72% carbohydrate, 11% protein, 17% fat) led to higher post-prandial leptin levels compared to lean controls over 3h, with no changes observed over time in either group (107). Though this suggests that leptin levels may not respond to caloric intake in the short-term, further studies are needed to confirm the satiety effects of leptin in children.

2.5 Puberty

Puberty results from the reactivation of the hypothalamic pulsatile secretion of gonadotropin-releasing hormone (GnRH). GnRH then activates the hypothalamic–pituitary–gonadal (HPG) axis. Activation leads to the release of pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and the subsequent production of sex steroids. This cascade of events is accompanied by the development of secondary sexual characteristics and significant physical maturational changes, including rapid linear growth and changes in the proportion of fat and fat free mass (108). There is a sexual dimorphism in body composition: during puberty, males acquire greater amounts of lean body mass, whereas females gain significantly more fat mass (109). Pubertal body composition may predict adult body composition (110) and negatively impact health.

Although the sequence of events is maintained, timing of pubertal onset differs in girls and boys. Onset in girls occurs between 8 and 12 years and boys 9 to 14 years of age. Girls attain a peak height velocity at an average 11.5 y (Tanner breast stages 2 and 3), while boys experience
peak height velocity from about 13.5 y of age (Tanner genital stages 3 and 4) (111). In a cohort of Danish children born between 1930 and 1969, there was a decline in the age at which the onset of pubertal growth spurt and age at peak height velocity occurred (112). The increase in childhood obesity may contribute to this trend. A recent 2012 review concludes that increasing BMI is positively associated with earlier pubertal development in girls, but that the evidence in boys is inconsistent (111). The mechanisms linking obesity, timing of puberty and progression of puberty require further investigation.

2.5.1 Hormonal Regulation of Food Intake During Puberty

Children’s FI patterns are concordant with sex-specific changes in body composition, peak growth velocity and pubertal development (113-115). In later puberty, FI is greater in males than in females (116). Furthermore, both males and females consume more calories in later compared to early puberty; however, increased FI during pubertal development is attenuated after controlling for body weight, fat mass and fat free mass (116).

Estrogen and testosterone are the primary sex steroids produced during pubertal development in girls and boys. The development of secondary sex characteristics, such as breast development in girls and testicular enlargement in boys, is the result of increased estrogen and testosterone production via gonadotropin secretion during puberty (117). In girls, estradiol is primarily produced from developing ovarian follicles (117). However, testosterone is also converted to estradiol via aromatization (118). In boys, testosterone, produced in the testes, is the principal male sex hormone (117).

Estrogen and testosterone affect FI and interact with appetite hormones. Estradiol has a suppressive effect on FI in adult rats (119, 120). Ovariectomized rats have greater FI, which can
be reversed by restoring physiological levels of estradiol (119). Energy intake in women is decreased in the follicular compared to the luteal phase of the menstrual cycle due to higher levels of estrogen (121). In contrast to estradiol, less is known about testosterone’s role on FI. Studies in adult rats show that testosterone has a stimulatory affect on FI (122) and that orchiectomy in adult male rats decreases food intake, an effect reversed by physiological doses of testosterone (123). The individual effects of the sex steroids on FI may be the result of their interaction with gastrointestinal peptides, but results comparing animal and humans are inconsistent. In rats, estradiol increases CCK’s satiating effects (124, 125) and attenuates ghrelin’s orexigenic effects (119), leading to lower FI. However, estrogen administration to short peripubertal girls (8 – 12.5 y), did not affect ghrelin or leptin levels (126). In the same study, testosterone administration to short peripubertal boys (8 – 12.5 y) leads to a marked decline in circulating levels of ghrelin and leptin (126). It is unclear whether physiological changes in sex hormone concentrations in children lead to alterations in FI during puberty.

The following discusses the hormonal levels of PYY, ghrelin, insulin and leptin and how they are altered during puberty. Although there are potentially many other hormones related to FI, these have been most studied in children and adolescents.

2.5.1.1 PYY

PYY is postulated to have a role in the regulation of the HPG axis, but the results are conflicting. In the anterior pituitaries of prepubertal rats, PYY(3-36) increases LH and FSH secretion in vitro, but not when delivered peripherally (127). However, central administration of PYY(3–36) in vivo inhibits LH secretion, whereas FSH levels were unaffected, in prepubertal
male, but not female rats (127). Furthermore, administration of growth hormone, which rises during puberty, results in reduced PYY concentrations in rodents (128).

Similar to rodents, a recent study in adolescents demonstrated an inverse relationship between growth hormone and PYY: when growth hormone levels are highest, PYY is at a nadir. This relationship was evident during mid-puberty in both boys (Tanner 3-4) and girls (Tanner 2-3) (129). Low PYY concentrations during early-mid puberty in girls and mid-late puberty in boys may increase energy intake during this period of growth (129). In the study described above, fasting concentrations of PYY were measured, so it remains unclear how PYY responds to macronutrient consumption by children during puberty. These findings, although sparse, implicate PYY in the regulation of the HPG axis and that lower fasting PYY is partly associated with pubertal progression.

2.5.1.2 Ghrelin

Ghrelin’s orexigenic effects have been largely studied, but evidence also suggests a role for ghrelin in modulating growth and development. Ghrelin is a potent releaser of growth hormone (130) and like leptin, is a pleotrophic regulator. Both ghrelin and receptors are expressed in the pituitary and hypothalamus (131). Ghrelin administration inhibits LH secretion in rats (either intact or gonadectomized) and humans (132-135) through a number of potential mechanisms, including i) stimulation of NPY, AgRP and orexins that have an inhibitory effect on the central control of the HPG axis; (ii) interaction and suppression of the opioid system to inhibit the stimulation of LH secretion (135); (iii) stimulation of corticotrophin releasing hormone which inhibits LH secretion (136) and (iv) stimulating prolactin secretion, which also inhibits gonadotropin release, as shown in adult humans (137).
Studies in rodents and humans have demonstrated ghrelin’s role in puberty initiation. Repeated injections of ghrelin in male rats during the pubertal transition decrease serum LH and testosterone levels, and partially delay balano-preputial separation (an external index of puberty onset) (132, 138). Studies in humans suggest a permissive role for ghrelin in pubertal maturation (139). In the first two years of extrauterine life, serum ghrelin levels increase with a concomitant decline in GnRH activity, followed by a decrease in ghrelin until pubertal completion (140, 141). These findings suggest an inverse relationship between activation of the HPG axis and ghrelin levels. Similarly, mean ghrelin concentrations in boys in Tanner stages 4 and 5 are about 40% lower compared to Tanner 1 and are inversely correlated with testosterone levels (142). A recent study showed that fasting levels of acylated and unacylated ghrelin are higher in pre-pubertal (Tanner I) compared to pubertal (Tanner II-V) NW and obese children and are inversely related to testosterone and estradiol (43). These findings support ghrelin’s role in pubertal onset and development. Only one study with a small sample size (n = 5-6 children/group) has examined ghrelin dynamics following an oral glucose load and its relationship to pubertal stages in children. Ghrelin was suppressed after an oral glucose load at 60 min in prepubertal, but not early pubertal boys (143).

2.5.1.3 Insulin

It is unclear whether insulin is a primary factor regulating puberty or a permissive factor like leptin. Less is known about the contribution of insulin on the neuroendocrine axis. Insulin and leptin interact at the hypothalamic level to downregulate NPY and AgRP and enhance POMC production and release (144). The central administration of insulin restores LH secretion
in diabetic rats (145), and insulin in vitro can stimulate both GnRH secretion and expression (146).

Insulin resistance is a physiological event during puberty. Fat mass accounts for a greater percentage of total body weight in girls compared to boys during puberty, and this change in body composition is associated with transient insulin resistance (147). Increased fat mass and BMI during puberty mediate changes in insulin (148); however insulin resistance can result independent of changes in BMI(149). A large cross-sectional study examining insulin resistance using euglycemic insulin clamps on 357 NW children (10–14 y), showed that insulin resistance increases by Tanner stage 2, remains stable between stages 2 and 4, and returns to approximate prepubertal levels by the end of puberty (Tanner 5). Changes in insulin were not associated with alterations in BMI or measures of adiposity, suggesting that changes in body composition are not the primary mediator behind pubertal insulin resistance. Overall, girls were more insulin resistant compared to boys, even after adjustment for differences in adiposity.

Increases in growth hormone and insulin-like growth factor 1 (IGF-1) during puberty (150) contribute to the heightened insulin resistance, and insulin resistance in adolescents is positively correlated with IGF-1 (149, 151, 152) and mean 24 h serum growth hormone levels (153). IGF-I levels in boys and girls closely mimic the rise and fall in insulin resistance during puberty (152). Increased insulin secretion is predicted to increase the availability of bioactive IGF-I to further promote growth during puberty (152).

Although pubertal insulin resistance is a normal physiologic process, it may increase the risk of developing impaired glucose tolerance or type 2 diabetes during adolescence (154) and exacerbate any underlying dysfunctional metabolic issues. In one longitudinal study insulin
resistance in males at age 11 y, increases steadily to 19 y, and did not increase in females (155). Increased insulin resistance in the older males is related to higher triglycerides and decreased high-density lipoprotein cholesterol at 19 y, despite lower body fat, and suggests that insulin resistance in males during young adulthood is implicated in the increased cardiovascular risk found in adult males (155).

2.5.1.4 Leptin

Leptin is involved in the integrated control of metabolism and pubertal development through its ability to modulate several neuroendocrine systems, including the HPG axis (156). Leptin suppresses NPY expression and secretion by neurons in the arcuate nucleus to modulate these systems (157). NPY can suppress growth hormone through stimulation of somatostatin (158), suppression of gonadotropins (158), or stimulation of the hypothalamic pituitary-adrenal axis (159). Therefore, leptin stimulates growth hormone secretion through suppression of NPY(160). In adult rat testis in vitro, leptin can directly act at the level of the gonads, and inhibit testosterone secretion(161).

There is no consensus as to whether leptin is a signal for the initiation of puberty or if a critical leptin level is necessary for onset of puberty. The administration of small doses of leptin in mice during early puberty encourage sexual maturation; this does not occur in prepubertal mice, suggesting that leptin is a permissive signal for pubertal development (162). However, a nocturnal increase in leptin secretion occurs before puberty in rats. This may implicate leptin as an initiator in the timing of puberty(163). In humans with leptin deficiency or leptin receptor
mutations, failure to initiate puberty is a hallmark characteristic, thus leptin may be necessary but not sufficient to regulate normal pubertal progression (164).

Initially, serum leptin levels are low in male and female prepubertal children (165-167). There is also a sexual dimorphism in leptin levels as puberty progresses. Leptin is higher in females compared to males, even after correcting for BMI. This dimorphism may be due to differences in body fat distribution or the effect of sex hormones (166, 168). In males, prior to elevations in testosterone, leptin increases until Tanner stages 2 to 3 and subsequently decreases as puberty advances (167), which may be explained by the suppressive effect of androgens (169). In contrast, estradiol is positively correlated with leptin levels (167), thus females demonstrate a continuous and progressive increase in leptin levels throughout puberty (170). This increase in leptin during puberty in females is consistent with an increase in body fat (171), suggesting that leptin may signal to the body that energy reserves are adequate for reproduction (172).

2.6 Environmental Factors Affecting Food Intake

The physiological regulation of FI in children can be overridden by a plethora of environmental factors which are correlated with risk for obesity. Non-food related factors such as sleep deprivation (173, 174), reduced physical activity (175, 176) alcohol consumption (173), smoking history (175), socioeconomic status (177) and mental illness (178, 179) can negatively impact body weight. Food-related environmental factors relate to the way food is provided or presented, such as its salience, variety, package or portion size, and palatability. In contrast, the mealtime environment refers to those factors associated with the eating of food, but that are
independent of food, how food is obtained, the eating atmosphere, the social interactions that occur with family, friends, and peers, and the distractions that may be taking place, including TVV (180). Non-food, food-related and mealtime factors may contribute to how much energy is consumed at a meal and can operate together to impact FI. However, the primary focus of this review is on the mealtime environment, specifically TVV.

A recent meta-analysis reported that TVV was not merely associated with obesity, but likely contributes to weight gain by facilitating excess FI (173). TVV affects cognitive functions involved in reward saliency and inhibitory control and is a common activity for children and adolescents. In children the relationship between food characteristics and satiety and satiation involves learning the associations between food and satiety signals (181); however, during puberty, both physiological and environmental factors may disrupt these learning associations leading to altered FI. TVV is a distraction that may disrupt or override satiety signals and increase FI in children during pubertal development. The following section discusses the relationship between TVV, body weight and appetite regulation in children and adolescents.

2.6.1 TVV

TVV is one of the most frequent leisure-time activities contributing to the obesity epidemic in both children and adults (16, 182-184). Excessive screen time among children is a growing public health issue as it can impact psychosocial and physical health (185-187). TVV is considered one of the most modifiable causes of obesity. Possible causes of the relationships between TVV and obesity are increased preference for energy dense foods and sweetened beverages (188), decreased resting metabolic rate (189), meal skipping with overeating later (190), distracted eating (191) and reduced activity (192) while TVV.
2.6.1.1 TVV and Body weight

Epidemiological studies indicate positive relationships between screen time and increased BMI in children and adolescents (16, 184). A longitudinal survey of adolescents (12 – 17 y) found that two or more hours of TVV at baseline was associated with a doubled probability of being overweight or obese at the 3 y follow up session, independent of baseline BMI (184). In this same age group, the UK’s National Health Examination survey indicated that each additional hour of TVV was associated with a 2% increase in the prevalence of obesity (16). Furthermore, hours of TVV was positively associated with prevalence of overweight and obesity in 6 to 11 y old children (16), further demonstrating that excessive TVV is a concern for youth of various ages. In a separate study, girls who watched 5 or more hours of TV/day were consuming, on average, an extra 175 kcal/day compared to non-frequent TV viewers and engaged in less physical activity compared to boys. Increased TVV was positively associated with obesity in girls, even after controlling for age, race/ethnicity, family income, weekly physical activity, and energy intake. (185). Additionally, TVV in early childhood is associated with increased risk of remaining obese into adulthood (193, 194). However, in these epidemiological studies, TVV was not directly measured and were derived from self-reported data completed by children and their parents. Furthermore, these findings only indicate associations and do not prove cause and effect relationships.

Results of trials to reduce TVV and BMI however, are disappointing. A systematic review and meta-analysis of randomized controlled trials that focused on interventions to reduce screen time failed to demonstrate the effectiveness of these trials on reducing BMI and screen time; however, a subgroup analysis of preschool children showed a difference in mean change in
screen time (195). The authors suggest a need for more rigorous investigations over shorter periods of time with longer follow-up, that focus on specific age groups, such as preschool children, where these interventions that focus on reducing screen time may be easier to follow.

2.6.1.2 TVV and Food Intake

Epidemiological studies suggest a positive correlation between hours of TVV and energy intake in adult women (196) and children (197). Television viewing during meals or snacks is a common occurrence and accounts for about a quarter of total daily energy intake (198). Furthermore, self-reported data demonstrated that increased TVV time was associated with higher snacking and lower intake of fruits and vegetables in 9 – 11 y old boys and girls (199). In adults, several clinical trials found that TVV that included no food cues increased energy intake from an ad libitum test meal (200-202). Individual adults watching an interactive game show while eating from a buffet meal increased their energy intake to a similar extent as eating with two friends compared to eating alone with no distraction (202). Similarly, in another clinical study, when undergraduate students ate alone while watching the TV show of their choice, their intake of high fat food was significantly higher compared to when they were listening to a pre-selected symphony (201).

In children and young adults, however, the effect of TVV on energy intake is not consistent and the results vary depending on age and sex of the subjects, the source of the energy provided and previous exposure to TV. Previous exposure to TV may influence later FI while TVV. Pre-school children (3-5 y) consumed less total weight of food from a snack and meal while TVV (203); however, those children who reportedly eat while watching TV at home ate more during the lunchtime TVV session versus children who rarely eat during TVV (203). In
contrast, in NW adolescents (15 - 16 y old), TVV did not affect energy intake from a buffet meal, increased soda intake compared to other conditions. However, due to the setting of the eating environment (eating in a room where TV was present as opposed to sitting directly in front of the TV) the subjects may not have paid attention to the TV program (204).

TVV as a distraction is known to be distracting and disrupt habituation to food cues, particularly when there is active engagement in the stimulus (attention allocation), leading to greater energy intakes. Intake from their favourite snack was higher in 9 to 12 y old children while watching a continuous TV show compared to a repeated segment of a TV show (205). Similarly, watching a non-food related TV show during the meal decreased both satiation at the test meal and satiety from the glucose drink, leading to higher energy intakes (206). Boys (9 – 14 y old) increased their FI at an ad libitum pizza meal by 24% while TVV and showed diminished response to satiety signals from a pre-meal glucose drink given 30 min before the pizza meal (206). However, how TVV affects satiety and FI in girls in this age range is unknown.

2.7 Food/Macronutrients and the Regulation of Food Intake in Children and Adolescents

While studies are continuing to emphasize the relationship between impairments in appetite hormones and FI in obese children, few studies have examined the role of macronutrients alone or in combination and their interaction with obesity, puberty and sex on FI and post-prandial hormone concentrations in children. This is important because satiety signals after macronutrient consumption in overweight/obese children may be dysregulated; however, an understanding of the role of body weight, pubertal stage and sex is needed when considering
factors regulating FI in adolescents. The following section will review FI regulation in children and adolescents as affected by macronutrient specific mechanisms of FI regulation.

Metabolic energy is ultimately derived from the three macronutrients: carbohydrate, fat and protein. Studies of regulation of FI in response to macronutrient ingestion are still relatively new and unexplored in children. Few studies have investigated intake regulation in children by measuring the effect of macronutrient preloads on caloric compensation at test meals. Caloric compensation is a term used to express the reduction (compensation) in caloric intake in a test meal in response to calorie-containing drinks or foods (the preloads). Some studies suggest that childrens’ compensation to caloric preloads consumed before test meals is highly variable and less precise in older compared to younger children (207). This variability may be due to the effect of sex and pubertal status, factors which have not been examined in the majority of FI studies in children. More research is needed on the physiological control of FI in children to determine whether short-term signals leading to increased satiety (less hunger) and satiation (termination of eating) are macronutrient sensitive.

Dietary components can interact with other physiological and environmental determinants of FI that lead to its dysregulation. All macronutrients provide energy; however, their effect on FI in children cannot be predicted from their caloric content alone. Each macronutrient exerts specific satiating effects independent of their caloric value (208). Dietary protein is a stronger contributor to short-term satiety than to carbohydrate or fat (209). Carbohydrates, protein and fat exert their individual satiating effects via the release of appetite-hormones from the gastrointestinal tract, pancreas and adipose tissue (210), as well as through other postingestive actions. Furthermore, macronutrient source is a factor affecting FI and appetite (211).
2.7.1 Carbohydrates

Carbohydrates are the primary macronutrient source of energy in most diets, averaging between 45 - 60% of total energy intake (212). Carbohydrate consumption increases blood glucose, which is associated with satiety and reduced FI. Short-term satiety is associated with the glycemic effects of carbohydrates, as high compared to low glycemic carbohydrates suppress FI more up to 2 h (213). However, in addition to glucose as a signal to the brain, hormones are involved. Release of insulin (48) and gut hormones, GLP-1 (214) and CCK (215) help to elicit carbohydrate-induced satiety by affecting gastric emptying rate and stimulating satiety regions in the CNS (48). Satiety signals arising from sugars are unique compared to those arising from other carbohydrates because sweet-tasting products lead to satiation in later meals (216).

2.7.1.1 The effect of carbohydrates on short-term food intake in children and adolescents

Compensation for carbohydrate energy given as solids is dependent on the quality and timing of the preload prior to measurement of FI in young children. Preschool children (2.5 – 5 y) compensated accurately at a lunch buffet given 20-30 min later for the energy content of pre-meal carbohydrate puddings of varying energy densities. Compensation was also greater in children compared to adults (217). However, children (4 – 6 y) failed to compensate at a lunch buffet after a high carbohydrate (82%) yoghurt preload when the time interval between the preload and test meal was extended to 90 min (218).
Sugars consumed as liquids do not bypass regulatory systems (219), as shown in studies investigating the effect of sugars on appetite and FI in children. In children (2 – 5 y), 90 kcal from a sucrose drink was sufficient to suppress intake at test meals 0, 30, or 60 min later. Caloric compensation at 30 min was 100%, indicating that young children are able to rely solely on internal hunger cues (220). Normal weight, pre-school children (3 – 5 y) also demonstrated almost perfect caloric compensation in a test meal given 30 and 60 min after preloads containing sucrose, low glucose maltodextrin or a combination of both (average of 1.25 g/ kg of body weight) (220-223). When older children (9–10 y) consumed a cherry-flavored drink containing either 45 or 90 g sucrose, compensation for the 45- and 90-g sucrose beverages was 68% and 63%, respectively and lunchtime FI was reduced 30 min later (224). However, sucrose failed to suppress FI in children of the same age (9-10 y) when the intermeal interval was 90 min (225), further supporting the importance of time to the next meal on compensation in children.

Few studies have examined the interaction between carbohydrate ingestion and obesity. Poorer self-regulation is associated with increased adiposity of the child (226, 227). Body fatness, based on skinfold measurement, was inversely associated with FI reduction at a later meal in 3- to 5- year old girls, but not in boys after consumption of beverages containing sucrose/low-glucose maltodextrin. Caloric compensation was greater in boys (55%) than in girls (35%), suggesting that the difference in compensation was likely attributed, in part, to a greater proportion of body fat in the girls(226). In a recent study, NW boys and obese boys (9 – 14 y) reduced their FI 30 min after consumption of a glucose drink (50 g), suggesting that satiety response to carbohydrates is not affected by obesity. FI was not suppressed in NW boys when measured 60 min after consumption of glucose (1.0 g/kg body weight) (228), but the response in the obese boys was not tested.
2.7.2 Protein

Dietary protein contributes more strongly to short-term satiety than carbohydrate or fat in adults as indicated by both quantitative and subjective measures (209). This is primarily due to the postigestive effects of protein and the mechanisms involved in protein-induced satiety (209). First, satiety is triggered by the digestion of protein and subsequent release of biologically active peptides. These peptides interact with CCK-A (229), opioid (230) and GLP-1 (214) receptors and provide signals via the vagus nerve either directly or by their interaction with appetite-hormones such as CCK, GLP-1, PYY and/or ghrelin (231). Furthermore, amino acids released from the digested protein into the blood can regulate FI by stimulating centres in the CNS (232-234) and enhancing satiety.

In adults, the effect of protein on FI is dependent on the source. However, the effect of source is modified by factors such as dose, form (solid vs. liquid), the presence of other macronutrients and time to the next meal (231). Whey protein (48 g) increased subjective appetite and decreased FI more than casein (48 g) at a buffet meal 90 min later in healthy adults (235). Furthermore, FI was lower after both whey and soy protein (45 g) beverages compared to egg albumin at a pizza meal measured 60 min later in young men. Whey, but not soy protein, decreased FI more than sucrose (236). Compared with casein, whey suppressed FI at 85, but not at 150 min (237), demonstrating that composition of the source determines the duration of effect, similar to carbohydrates.
2.7.2.1 The effect of protein on short-term food intake in children and adolescents

Compared to carbohydrate, little is known about the effect of protein on FI in children. When young children (5 – 6 y) were given a high carbohydrate (~67 g carbohydrate) or a high protein (~46 g protein) meal, they consumed more energy from the high carbohydrate meal. At a subsequent meal 3.5 h later, children consumed less energy after the high protein meal, demonstrating a greater effect of protein on satiety and satiation compared to carbohydrate (238).

There is only one study that has looked at the effect of a pure protein source on FI in children. Normal weight boys (9 – 14 y) given a 50 g WP drink suppressed FI at a pizza meal 30 min later, but not more than a glucose (50 g) drink. However, WP (1.0 g/kg body weight) reduced FI in NW boys at 60 min, demonstrating that as in adults, the effect of WP is both dose and time-dependent. In obese boys of the same age, 50 g of WP did not suppress FI at a pizza meal 30 min later (228). Differences between NW and obese boys in their response to WP suggest that body weight is a factor affecting FI in children.

2.7.3 Fat

Fats, depending on chain-length and degree of saturation, stimulate satiety responses, including GLP-1 (239), CCK (240) and delay gastric emptying (240, 241). However, fats are less satiating than proteins and carbohydrates. Due to their higher caloric value, fats may contribute less in the regulation of FI in terms of maintaining energy balance (242). Examining the short-term effects of fat on FI is important because both children and adults prefer and choose energy-dense foods that are richer in fat (243). Furthermore, the mu-opioid system is a key target for the
hedonic experience of feeding and mu opioid receptor stimulation of the nucleus accumbens increases the intake of and preference for high fat foods (244).

Fat preloads have less effect on short-term FI than carbohydrates in young adults at a meal (245). Only the largest dose (1254 kJ) of safflower oil suppressed intake, but was not consistent across repeated experiments. These results suggest that greater amounts of fat must be consumed before an effect on FI is observed. In another study, young males did not decrease FI 70 min after consuming 240 kcal of corn oil (246) but a large dose of 500 kcal (2090 kJ) of Intralipid infused into the stomach of young males suppressed FI 30 min later (247). There are no other reports on the relationship between the pure fat sources and subsequent FI.

2.7.3.1 The effect of fat on short-term food intake in children and adolescents

Although fat reduces FI to a lesser extent than carbohydrate or protein (210), the few studies looking at fat consumption in young children have shown that there is a suppression in short-term FI. Preschool children (2 – 5 y) show excellent compensation for energy density differences produced by manipulating the fat content of ice cream (248), yogurt (249) or by substitution of dietary fat by a non-energy fat substitute (250). However, children (4 – 6 y) did not compensate at a lunch buffet 90 min after consumption of a high fat (71%) yoghurt preload(218). While there is no information on the response of older children or obese children to fat, obese children have a preference for fatty foods (251, 252), suggesting that the response to the satiating properties of fat is attenuated.
2.8 Summary and Research Rationale

Childhood obesity remains a growing concern, yet little is known about the physiological factors affecting the regulation of FI during the development of puberty. Puberty is a period of growth associated with alterations in energy intake in both males and females, and how this period interacts with other physiological and environmental factors to affect short-term FI has not been studied. This is important as obesity during childhood and adolescence increases the risk of adult obesity.

The evidence presented shows that FI regulation is different in children than in adults and that it is affected by obesity, the macronutrient composition of food and distraction (TV viewing). However, the interactions between these factors and pubertal stage on FI control have not been reported. Although the prevention and management of obesity is proposed to begin in childhood, little is also known about FI regulatory hormone responses to macronutrient ingestion between boys and girls of varying body weights and during the development of puberty. While boys respond differently to pre-meal drinks containing carbohydrate and protein, it is unclear whether pubertal status affects this response. Furthermore, if pubertal status does affect compensation to pre-meal caloric drinks, what happens when distraction is added to the mealtime environment?

Therefore, the objective of this research is to develop an understanding of physiologic and environmental variables and how they interact to affect appetite and short-term FI regulation.
in children and adolescents during puberty. The results will emphasize how FI is regulated during this critical stage of development and ultimately assist with managing overconsumption.

3 HYPOTHESES AND OBJECTIVES

3.1 General Hypothesis and Objective

Hypothesis

• Physiological and environmental variables are both independent and interactive in determining food intake in children and adolescents during puberty.

Objectives

• To understand how physiologic and environmental variables affect appetite and short-term FI regulation in children and adolescents during puberty.

3.2 Specific Hypotheses and Objectives

Chapter 4: OBESITY, SEX AND PUBERTAL STATUS AFFECT GLYCEMIC AND APPETITE HORMONE RESPONSES TO A MIXED GLUCOSE AND WHEY PROTEIN DRINK IN ADOLESCENTS (submitted to Clinical Endocrinology, Manuscript# CEN-2013-000784).

Hypothesis:

• Appetite hormones at fasting and in response to a mixed drink (glucose (30 g) and whey protein (WP, 30 g)) are affected by obesity, sex and pubertal status.

Objective:
• To examine the effect of obesity, sex and pubertal status on subjective appetite and appetite hormones in response to a mixed glucose and WP drink in children and adolescents (8 – 18 y old).

Preface:

This chapter addressed the hypothesis that appetite hormones at fasting and in response to a mixed drink (glucose (30 g) and whey protein (WP, 30 g)) are affected by obesity, sex and pubertal status.

Chapter 5: PUBERTAL STATUS AFFECTS CHILDREN’S FOOD INTAKE RESPONSE TO CARBOHYDRATE, BUT NOT PROTEIN DRINKS (submitted to Clinical Nutrition, Manuscript# YCLNU-D-13-00429).

Hypothesis:

• Puberty affects compensation for energy intake in beverages.

Objective:

• To examine the effect of pubertal status on subjective appetite and FI following consumption of glucose and WP drinks in male and female children and adolescents.

Preface:

This chapter addressed the hypothesis that puberty affects compensation for energy intake in glucose and whey protein beverages.


Hypothesis:
• Television viewing reduces the effect of subjective feelings of satiety arising from a glucose drink and increases mealtime FI in peripubertal and postpubertal girls.

Objective:

• To examine the effect of television viewing while eating and pubertal status on subjective appetite and mealtime FI 30 min after consumption of glucose (1.0 g/kg body weight) or noncaloric sweetened drink in girls.

Preface:

This chapter addressed the hypothesis that television viewing reduces the effect of subjective feelings of satiety arising from a glucose drink and increases mealtime food intake in peripubertal and postpubertal girls.
4 Obesity, sex and pubertal status affect glycemic and appetite hormone responses to a mixed glucose and whey protein drink in adolescents

4.1 ABSTRACT

**Background and Objectives:** Little information is available on how food intake regulatory hormones may be altered during pubertal development and across the weight spectrum in adolescents. Therefore, the effect of obesity, sex and pubertal status on subjective appetite and appetite hormones in response to a mixed glucose and whey protein drink was determined in 8-18 y old adolescents.

**Patients and Methods:** A cross-sectional cohort study was conducted at the Hospital for Sick Children, Toronto. After a 12 h fast, normal weight (n = 5F, 4M) and obese (n = 5F, 4M) adolescents (Experiment 1), and pre-early pubertal (n = 10) and mid-late pubertal (n = 10) obese male adolescents (Experiment 2) consumed a 250 mL glucose (30 g) and whey protein (30 g) beverage. Insulin, PYY, ghrelin and subjective appetite were measured over 120 min.

**Results:** Obese adolescents (Experiment 1) have higher insulin, PYY and lower ghrelin (p < 0.006) than normal weight controls, with a more pronounced effect in males (p < 0.037). Puberty (Experiment 2) did not affect insulin (p = 0.305), but the change in PYY in response to the drink was greater (p = 0.032) and ghrelin was lower (p = 0.026) in mid-late pubertal than pre-early pubertal obese males. Average appetite 60 min post-drink was higher in obese and mid-late pubertal adolescents, but not related to hormone changes.
**Conclusions:** Obesity, sex and pubertal status affect macronutrient-stimulated appetite hormone secretion and these factors may alter food intake in obese children during pubertal development.

*This work was submitted to Clinical Endocrinology (Manuscript# CEN-2013-000784).*

### 4.2 INTRODUCTION

Prevention/management of obesity is proposed to begin in childhood (253), yet there is little information on how appetite hormones may be altered during pubertal development and across the weight spectrum. Furthermore, evidence available is contradictory. Fasting anorexigenic peptide YY (PYY) concentrations are found to be similar (45, 47, 79, 254), higher (54) or lower (88) in overweight/obese than normal weight (NW) children/adolescents (7-18 y). Fasting concentrations of orexigenic ghrelin, which are elevated pre-meal and decrease postprandially (95), are found to be lower in obese (43-47) in most, but not all studies (54, 79), than NW children. In response to a mixed meal (47, 79) or oral glucose load (46), PYY (46, 47) does not increase, while ghrelin fails to decrease (46, 79) in obese compared to NW children. Ghrelin concentrations may also be sex-dependent; median non-fasting ghrelin is similar between males and females (5-18 y) (141), while mean post-meal ghrelin concentrations were higher in NW and obese (6-12 y) females than males(107), although other studies have found no sex differences (255, 256).

Most studies have described appetite hormone responses in prepubertal children, but puberty is a time of rapid growth associated with increased food intake (FI) and altered body composition. Leptin decreases in males and increases throughout puberty in females (170). Insulin resistance is a normal physiological event occurring during mid-puberty; females are more insulin resistant than males (147). Gut hormones may also change, but little is known
during this developmental period (156). No studies have examined the interaction between obesity and appetite hormones with consideration of pubertal stage in boys and girls in response to a mixed drink. Understanding how puberty may alter hormonal response in NW and overweight children is of interest from a physiologic viewpoint, but may provide the rationale for targeted treatment interventions to increase satiety in overweight/obese children and adolescents.

We hypothesized that appetite hormones at fasting and in response to a mixed glucose and whey protein (WP) drink are affected by obesity, sex and puberty. In Experiment 1, the drink was given to NW and obese male and female adolescents to examine the effect of obesity and sex. In Experiment 2, the drink was given to pre-early pubertal (PEP) and mid-late pubertal (MLP) obese males to examine the effect of puberty. Subjective appetite, blood glucose, insulin, PYY, and ghrelin were measured.

4.3 MATERIALS AND METHODS

4.3.1 Subjects

Eleven to eighteen year old participants were recruited through newspaper advertisements.(228, 257) Exclusion criteria included physical/intellectual limitations precluding participation, medications interfering with weight/growth (eg, corticosteroids) and chronic illness (e.g. diabetes). Written informed assent was obtained from participants and authorization from legal guardians. A pediatric endocrinologist examined all participants to assess their pubertal status using the Tanner method (258). PEP and MLP participants were categorized as Tanner stages I-II (pre-early) and III-V (mid-late), respectively. Participants’ heights (m) were measured using a stadiometer and weight (kg) was recorded from a digital scale. Age-and-sex specific BMI
percentiles were calculated using WHO growth charts (9). Body composition was estimated using bioelectrical impedance analysis (RJL Systems BIA, 101Q) based on the Horlick equation (259), as previously described (228, 257). Insulin sensitivity was assessed one month earlier using a multiple sampled 2 h oral glucose tolerance test [1.75 g/kg body weight (up to 75 g)]. Whole body insulin sensitivity index (WBISI) uses the following formula: \[
WBISI = \frac{10,000}{\text{fasting glucose} \times \text{fasting insulin} \times \text{mean glucose concentration} \times \text{mean insulin concentration}}^{1/2}
\]. WBISI encompasses both hepatic and peripheral tissue insulin sensitivity (260), and is reported to be lower in obese individuals and decreases from pre- to mid-puberty (147). The Research Ethics Board at The Hospital for Sick Children (Toronto, ON) approved the study.

4.3.2 Experimental Procedure

After a 12 h fast, participants arrived for their one visit at the Physiological Research Unit, The Hospital for Sick Children at 0800 and were interviewed for compliance. A motivation-to-eat visual analogue scale (VAS), measuring dimensions of subjective appetite, was administered. A composite score of the 4 appetite questions in the VAS was calculated to obtain average appetite as previously reported (228, 257). Participants were asked to fully consume (within ~5 min) a drink containing 30 g glucose monohydrate (Grain Process Enterprises, Toronto, Canada) and 30 g plain WP isolate (Interactive Nutrition International Inc., Ottawa, Canada) plus aspartame-sweetened, orange-flavored crystals (1.1 g, Sugar Free Kool-Aid, Kraft Canada Inc., Don Mills, Canada) to standardize flavor, followed by 50 mL of water to minimize aftertaste. The rationale for the mixed beverage was based on the hypothesis that protein and carbohydrate combined would provide a more sustained satiety hormone response than each
alone (236) and the dose of 30 g of each was based on previous studies showing a reduction in FI in adults after pre-meal consumption of either ~25 g of sucrose (261) or 20-40 g of WP (262). The drink was prepared 1 day prior to consumption in a covered, opaque cup and stored in the refrigerator. Blood was drawn at 0, 15, 30, 60, 90 and 120 min post-treatment using an indwelling intravenous line placed by an experienced nurse for phlebotomy into pre-chilled tubes for glucose, insulin, PYY and ghrelin. Appetite was assessed before blood was drawn.

4.3.3 Biochemical assays

Plasma glucose was analyzed using the Ortho Clinical Diagnostics Vitros 950 analyzer and plasma insulin was analyzed by chemiluminescence immunoassay (Siemens Immulite 2500 analyzer). Total plasma PYY (intraassay CV, 0.9-5.8%; sensitivity, 6.5 pg/ml) that detects both the cleaved form (PYY3-36) and full-length hormone (PYY1-36) and acylated ghrelin (intraassay CV, 0.9-7.5%; sensitivity, 15 pg/ml) were measured using ELISA (Millipore, St. Charles, Missouri). At the time of sample collection, aprotinin (Sigma-Aldrich, 1 mg/mL) was added to tubes for PYY assessment; phenylmethanesulfonylfluoride (Sigma-Aldrich, 10 mg/mL) was added to tubes for ghrelin assessment and subsequently acidified with 1.0N HCl (50 mg/mL). Samples were stored at -80°C until analysis.

4.3.4 Statistical analyses

Statistical Analysis Software version 9.2 (SAS Institute Inc., Carey, NC) was used. Two-tailed tests were used to determine differences in baseline characteristics between groups in each experiment. In both experiments, all dependent measures (glucose, insulin, PYY, ghrelin) were
normalized if necessary using log transformation. A 3-way repeated measures ANOVA (Proc Mixed) was used to analyze the effects of obesity (NW vs. obese), sex, time and their interactions (Experiment 1) and a 2-way repeated measures ANOVA was used to analyze the effects of puberty (PEP vs. MLP), time and their interaction (Experiment 2) on all dependent measures. AUC (calculated using the trapezoid rule) for each dependent measure was assessed using obesity, sex and an obesity-by-sex interaction (Experiment 1). Because we hypothesized *a priori* that PYY and ghrelin would be higher and insulin lower in PEP males based on previous studies,(43, 129, 147) one-tailed t-tests were used to analyze AUC differences between PEP and MLP, and two-tailed t-tests were used for glucose (Experiment 2). Change from baseline average appetite was assessed using time, obesity, sex and a time-by-obesity interaction (Experiment 1), and using time, puberty and a time-by-puberty interaction (Experiment 2). Tukey-Kramer post-hoc tests were performed when more than 2 groups were significant in Experiment 1, otherwise unpaired t-tests were used for post-hoc analysis. Conversion from metric to SI units for ghrelin and PYY are as follows: ghrelin: picograms/milliliter x 0.296 = picomoles/liter; PYY: picograms/milliliter x 0.25 = picomoles/liter.

Average appetite was calculated at each time of measurement by the formula: Appetite score = \[\text{desire-to-eat} + \text{hunger} + (100 – \text{fullness}) + \text{prospective food consumption}] / 4,\] which reflects the 4 questions on the motivation-to-eat VAS.(228, 257) Data are means ± SEM. Significance was considered at p < 0.05. Correlations between dependent measures were conducted using Pearson correlation coefficients.
4.4 RESULTS

4.4.1 Experiment 1: Effect of obesity and sex on glycemic and appetite hormone responses in adolescents.

4.4.2 Subject characteristics

Eighteen post-pubertal adolescents (14 – 18 y old) participated (Table 4.1). Nine (4M, 5F) were < 85th (NW) and nine (4M, 5F) were ≥ 97th (obese) age- and sex- specific BMI percentiles (9). Insulin sensitivity (WBISI) was higher in NW compared to obese adolescents (Table 4.1).

Table 4.1: Baseline characteristics of test subjects

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td></td>
<td>Normal weight (n=9)</td>
<td>Obese (n=9)</td>
<td>Pre-Early Pubertal (n=10)</td>
<td>Mid-late Pubertal (n=10)</td>
</tr>
<tr>
<td></td>
<td>Males (n=4)</td>
<td>Females (n=5)</td>
<td>Males (n=4)</td>
<td>Females (n=5)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>15.9 ± 0.7</td>
<td>16.1 ± 0.6</td>
<td>15.9 ± 0.8</td>
<td>16.1 ± 0.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60.1 ± 6.5</td>
<td>55.2 ± 3.7</td>
<td>106.9 ± 19.1*</td>
<td>108.7 ± 12.6*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.8 ± 7.5</td>
<td>164.0 ± 1.6</td>
<td>162.3 ± 2.3</td>
<td>163.7 ± 2.4</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>47.0 ± 12.3</td>
<td>47.9 ± 11.4</td>
<td>98.5 ± 0.7*</td>
<td>98.2 ± 0.8*</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>6.9 ± 4.3</td>
<td>12.3 ± 1.3</td>
<td>50.1 ± 16.7*</td>
<td>55.9 ± 9.5*</td>
</tr>
<tr>
<td></td>
<td>Normal Weight</td>
<td>Obese</td>
<td>Male</td>
<td>Female</td>
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<tr>
<td><strong>Fat-free mass (kg)</strong></td>
<td>52.1 ± 6.0</td>
<td>42.6 ± 3.0</td>
<td>55.3 ± 4.3</td>
<td>52.1 ± 3.2</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td>4.4 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Fasting insulin (pmol/L)</strong></td>
<td>27.5 ± 13.2</td>
<td>21.5 ± 5.6*</td>
<td>161.7 ± 68.8*</td>
<td>54.5 ± 18.1*</td>
</tr>
<tr>
<td><strong>Fasting total PYY (pg/mL)</strong></td>
<td>77.0 ± 5.9</td>
<td>59.8 ± 12.7*</td>
<td>160.6 ± 12.4*</td>
<td>90.8 ± 15.7*</td>
</tr>
<tr>
<td><strong>Fasting active ghrelin (pg/mL)</strong></td>
<td>480.6 ± 90.3</td>
<td>221.8 ± 51.9</td>
<td>137.0 ± 14.1*</td>
<td>124.2 ± 27.0*</td>
</tr>
<tr>
<td><strong>WBISI</strong></td>
<td>13.3 ± 2.8</td>
<td>9.6 ± 2.1</td>
<td>3.3 ± 1.3*</td>
<td>7.2 ± 2.8*</td>
</tr>
</tbody>
</table>

1Data are presented as means ± SEM. * A 2-factor ANOVA was used with body weight and sex as main factors and a body weight by sex interaction. *Significantly different from normal weight, #significantly different from males, p < 0.05.

2Significantly different from mid-late pubertal p < 0.001, unpaired t-test.

*Body composition determined from Bioelectrical Impedance Analysis based on the Horlick equation (22).

3Whole body insulin sensitivity index (23)

Table 4.2: Post-treatment area under the curve (AUC) means for blood glucose and appetite hormones

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight</th>
<th>Obese</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Glucose</strong> (mmol · min/L)</td>
<td>525 ± 45</td>
<td>531 ± 25</td>
<td>595 ± 33</td>
<td>546 ± 16</td>
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<td></td>
</tr>
<tr>
<td><strong>Insulin</strong> (nmol · min/L)</td>
<td>19.8 ± 3.5a</td>
<td>24.2 ± 5.0a</td>
<td>103.0 ± 25.8b</td>
<td>27.8 ± 9.6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total PYY</strong> (ng · min/mL)</td>
<td>9.5 ± 1.1</td>
<td>7.3 ± 1.1</td>
<td>20.6 ± 3.5*</td>
<td>15.9 ± 3.4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Active ghrelin</strong> (ng · min/mL)</td>
<td>16.9 ± 4.1</td>
<td>31.9 ± 6.4*</td>
<td>10.5 ± 1.6*</td>
<td>10.2 ± 2.0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pubertal stage</td>
<td>Pre-early puberty</td>
<td>Mid-late puberty</td>
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<tr>
<td>Glucose(^8) (mmol · min/L)</td>
<td>578 ± 32</td>
<td>553 ± 30</td>
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<tr>
<td>Insulin(^9) (nmol · min/L)</td>
<td>45.8 ± 6.9</td>
<td>51.4 ± 8.4</td>
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<tr>
<td>Total PYY(^{10}) (ng · min/mL)</td>
<td>18.1 ± 1.8(^*)</td>
<td>12.6 ± 2.2</td>
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<tr>
<td>Active ghrelin(^{11}) (ng · min/mL)</td>
<td>14.9 ± 2.3(^*)</td>
<td>10.9 ± 0.8</td>
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\(^1\)Data are means ± SEM; AUC, area under the curve.

\(^2\)**Experiment 1:** (n = 9 NW; 4M, 5F and n = 9 OB; 4M, 5F). A 2-factor ANOVA was used with body weight and sex as main factors and a body weight by sex interaction. \(^3\)Significantly different from normal weight, p < 0.05. Means with different superscripts are significantly different, p < 0.05.

\(^4\)Glucose AUC was not affected by body weight (p = 0.174) or sex (p = 0.480), and there was no body weight by sex interaction (p = 0.371).

\(^5\)Insulin AUC was affected by body weight (p = 0.005) and sex (p = 0.015) and there was a body weight by sex interaction (p = 0.009).

\(^6\)PYY AUC was affected by body weight (p = 0.001), but not sex (p = 0.065) or a body weight by sex interaction (p = 0.381).

\(^7\)Ghrelin AUC was affected by body weight (p = 0.007), but not sex (p = 0.116) or a body weight by sex interaction (p = 0.105).

\(^8\)**Experiment 2:** (n = 10 Pre-early and n = 10 Mid-late). *Significantly different from mid-late pubertal, unpaired one-tailed t-tests, p < 0.05. \(^9\)Glucose (p = 0.754) and \(^{10}\)insulin (p = 0.336) AUCs were not affected by pubertal status, but \(^{10}\)PYY (p = 0.040) and \(^{11}\)ghrelin (p = 0.033) AUCs were greater in pre-early compared to mid-late pubertal obese males.
4.4.3 Plasma glucose and insulin

Fasting glucose was not different between NW and obese adolescents (Table 4.1, p > 0.05). Glucose response to the beverage (0-120 min) was affected by time (p < 0.001), but not sex (p = 0.245) or obesity (p = 0.456), with no interactions amongst factors (p > 0.05). Overall mean glucose concentrations peaked at 30 min (5.7 ± 0.2 mmol/L) and returned to baseline by 120 min. Glucose AUC (0-120 min) was not affected by sex (p = 0.480) or obesity (p = 0.174, Table 4.2).

Fasting insulin was ~4.6-fold higher in obese than NW adolescents (Table 4.1, p < 0.001). Insulin response to the beverage (0-120 min) was affected by time (p < 0.001), sex (p = 0.036), and obesity (p = 0.005), however this relationship was primarily driven by obese males. Overall mean insulin concentrations peaked at 30 min (701 ± 163 pmol/L) and remained elevated over 2 h. Mean insulin concentrations and insulin AUC (0-120 min, Table 4.2) were ~2-fold higher in males than females and ~3-fold higher in obese than NW adolescents (p < 0.05). A sex-by-obesity interaction (p = 0.024, Figure 4.1) revealed higher mean insulin in obese males than NW males (5-fold) and females (4-fold) and obese females (3-fold, p < 0.024).
Figure 4.1: Experiment 1: Interaction between sex and body weight on mean insulin concentrations over time. (3-way ANOVA; time, p < 0.001; sex, p = 0.036; obesity, p = 0.005; sex-by-obesity interaction, p = 0.024; other interactions, p > 0.05. *Obese males were significantly different from all other groups (Tukey–Kramer post-hoc test, p < 0.05) (n = 9 NW; 4M, 5F and n = 9 OB; 4M, 5F). Data are means ± SEM.
4.4.4 Plasma PYY and Ghrelin

Fasting PYY was 1.8-fold higher in obese than NW adolescents (Table 4.1, p < 0.001). PYY response to the beverage (0-120 min) was affected by time (p = 0.008), sex (p = 0.035) and obesity (Figure 4.2a, p = 0.002), and there were no interactions. When differences in baseline were corrected by calculating the percent change of PYY, time, sex, obesity and the interactions among them were not significant (Figure 4.2b, p > 0.05). Mean PYY concentrations were 45% greater in males than females and 2-fold greater in obese than NW adolescents. Because there was a trend for a sex-by-time interaction (p = 0.056), a 2-way ANOVA was performed within each sex. PYY concentrations were ~2-fold higher in obese males and females than their NW counterparts (Figure 4.2c,d, p < 0.038). Time (p = 0.012) affected PYY only in females, increasing above baseline at 15 min (108.7 ± 26.4 pg/mL) and remaining elevated over 2 h. PYY AUC (0-120 min, Table 4.2) was 2.2-fold higher in obese than NW adolescents (p = 0.001), with no sex effect (p = 0.065).

Fasting ghrelin was ~72% lower in obese than NW adolescents (Table 4.1, p < 0.001). Ghrelin response to the beverage (0-120 min) was affected by time (p < 0.001) and obesity (p = 0.001), but not sex (p = 0.149). Both groups reached a nadir at 60 min, but the drop in ghrelin was greater in NW than obese adolescents (Δ0-60min: -207 vs. -59 pg/mL, p = 0.039). Mean ghrelin concentrations and ghrelin AUC (0-120 min, Table 4.2) were ~2.5-fold greater in NW than obese adolescents (p < 0.05). A time-by-obesity interaction (p = 0.018, Figure 4.2e)
demonstrated blunted ghrelin concentrations in obese adolescents at 0, 15, 60, 90 and 120 min post-drink. When differences in baseline were corrected by calculating the percent change of ghrelin, concentrations were reduced by the beverage at 30 min in NW adolescents only (Figure 4.2f, p = 0.032).
Figure 4.1: Experiment 1: Effect of obesity on (a) total PYY concentrations over time, (b) percent change in total PYY concentrations over time, (c) mean total PYY concentrations in males and (d) females over 120 min and on (e) active ghrelin concentrations over time and (f) percent change in active ghrelin over time. (a) Obesity (p = 0.002), time (p = 0.008), and sex (p = 0.035) affected PYY concentrations over time, with no interactions. (b) No factors affected
percent change of PYY (p > 0.05). (c, d) Body weight affected total PYY concentrations in males and females (p < 0.038), but only time affected total PYY in females (p = 0.012). §Significantly different from normal weight (Two-way ANOVA, p < 0.05) (n = 8 Males; 4 NW, 4 OB; and n = 10 Females; 5 NW, 5 OB). (e, f) *Significantly different within a timepoint (unpaired t-test, p < 0.05). (n = 9 NW; 4M, 5F and n = 9 OB; 4M, 5F). Data are means ± SEM.

4.4.5 Average appetite scores

There were no sex differences, so to examine the effect of obesity on post-treatment average appetite the results were pooled for sex. Average appetite was not affected by time or obesity (p > 0.05). A time-by-obesity interaction (Figure 4.5, p = 0.023) showed higher scores (greater appetite) at 60 min in obese compared to NW adolescents (p = 0.036). There were no correlations between any of the hormones and average appetite (data not shown).

Figure 4.3: Interaction between body weight and time (Experiment 1, a) and between pubertal status and time (Experiment 2, b) on average appetite. *Significantly different within a timepoint (unpaired t-test, p < 0.05). (Experiment 1: n = 9 NW; 4M, 5F and n = 9 OB; 4M, 5F, Experiment 2: n = 10 Pre-early and n = 10 Mid-late). Data are means ± SEM.
4.4.6 Associations between hormones and other dependent variables

For all subjects, fasting insulin correlated positively with fasting PYY ($r = 0.748, p = 0.001$), body weight ($r = 0.688, p = 0.003$) and fat mass ($r = 0.635, p = 0.008$), and negatively with fat free mass ($r = -0.634, p = 0.008$) and WBISI ($r = -0.747, p = 0.001$) and there was a trend for an inverse relationship with fasting ghrelin ($r = -0.462, p = 0.083$). Fasting PYY correlated positively and negatively with the same variables as insulin. Fasting ghrelin correlated negatively with body weight ($r = -0.643, p = 0.005$), fat mass ($r = -0.561, p = 0.019$) and fat free mass ($r = -0.611, p = 0.009$) and there was a trend for a positive association with WBISI ($r = 0.462, p = 0.072$). In the obese, but not NW group, only fasting insulin was negatively correlated with WBISI ($r = -0.740, p = 0.036$).

4.4.7 Experiment 2: Effect of pubertal status on glycemic and appetite hormone responses in obese males.

4.4.8 Subject characteristics

Twenty obese males participated (Table 4.1). Ten were pre-early pubertal (Tanner stage I-II) and ten were mid-late pubertal (Tanner III-V). WBISI was not different between pre-early pubertal and mid-late pubertal adolescents.
4.4.9 Plasma glucose and insulin

Fasting glucose (Table 4.1) and glucose AUC (0-120 min, Table 4.2) were not different between pre-early pubertal and mid-late pubertal obese males (p > 0.05). Glucose response to the beverage (0-120 min) was affected by time (p < 0.001), but not pubertal status (p = 0.259) or interaction (p = 0.860). Overall mean glucose concentrations peaked at 30 min (6.4 ± 0.1mmol/L) and returned to baseline by 120 min.

Fasting insulin (Table 4.1) and insulin AUC (0-120 min, Table 4.2) were not different between pre-early pubertal and mid-late pubertal obese males (p > 0.05). Insulin response to the beverage (0-120 min) was affected by time (p < 0.001), but not pubertal status (p = 0.305) or interaction (p = 0.243). Overall mean insulin concentrations peaked at 30 min (1052 ± 134 pmol/L) and remained elevated above baseline at 120 min.

4.4.10 Plasma PYY and Ghrelin

Fasting PYY (Table 4.1) and PYY AUC (0-120 min, Table 4.2) were ~1.5-fold higher in pre-early pubertal than mid-late pubertal obese males (Table 4.1, p < 0.05). PYY response to the beverage (0-120 min) was affected by time (p < 0.001) and there was a trend for an effect of pubertal status (p = 0.082), with 33% greater PYY in pre-early pubertal than mid-late pubertal obese males. A time-by-pubertal status interaction (Figure 4.4, p = 0.007) showed that pre-early pubertal males had higher PYY concentrations at 0, 15, 60 and 120 min than mid-late pubertal obese males. However, when differences in baseline were corrected by calculating the percent change of PYY, there was an effect of pubertal status (p = 0.032), with a greater mean PYY
response in mid-late pubertal (43 ± 7 %) than pre-early pubertal (6.2 ± 6.4 %) obese males (Figure 4.4, p = 0.032).

Figure 4.4: Experiment 2: Effect of pubertal status on (a) total PYY concentrations over time and (b) percent change over time. *Significantly different within a timepoint (unpaired, one-tailed t-test, p < 0.05). (n = 10 Pre-early and n = 10 Mid-late). (b) There was a significant effect of pubertal status (p = 0.032) only. Data are means ± SEM.
Fasting ghrelin was 1.7-fold higher in pre-early pubertal than mid-late pubertal obese males (Table 4.1, p < 0.001). Ghrelin response to the beverage (0-120 min) was affected by time (p = 0.003) and pubertal status (p = 0.026), but no interaction (p = 0.465). Both groups reached a nadir at 60 min (pre-early pubertal: 119 ± 20 pg/mL, mid-late pubertal: 82 ± 13 pg/mL) and the drop in ghrelin was lower in the mid-late pubertal than pre-early pubertal obese males (Δ0-30min: -41 vs. -109 pg/mL, p = 0.025). Mean ghrelin concentrations (Figure 4.5) and AUC (Table 4.2, p = 0.033) were ~1.5-fold higher in pre-early pubertal than mid-late pubertal obese males.

Figure 4.5: Experiment 2: Effect of pubertal status on active ghrelin concentrations over 120 min. *Significantly different from pre-early puberty (Two-way ANOVA, pubertal status p =
0.026, time p = 0.003, interaction p = p > 0.05) (n = 10 Pre-early and n = 10 Mid-late). Data are means ± SEM.

4.4.11 Average appetite scores

Average appetite was affected by time (p < 0.001), but not pubertal status (p = 0.209). A time-by-pubertal status interaction (Figure 4.3, p = 0.018) showed higher scores at 60 min in the mid-late pubertal than pre-early pubertal adolescents (p = 0.036). There was a trend (p = 0.074) for lower appetite AUC in PEP (-123 ± 387 mm · min) compared to MLP males (845 ± 507 mm · min). There were no correlations between any of the hormones and average appetite (data not shown).

4.4.12 Associations between hormones and other dependent variables

For all subjects, fasting insulin correlated positively with body weight (r = 0.507, p = 0.032) and fat mass (r = 0.525, p = 0.044) and negatively with WBISI (r = -0.583, p = 0.029). In the mid-late pubertal, but not pre-early pubertal, males, only fasting insulin correlated positively with fat mass (r = -0.685, p = 0.042) and negatively with WBISI (r = -0.921, p = 0.001). Fasting PYY and ghrelin were not associated with any of the dependent measures.

4.5 DISCUSSION
Appetite hormone responses to a mixed glucose and WP drink in adolescents are altered by obesity and by sex (Experiment 1). The differences observed between obese and NW males and females include higher insulin and PYY secretion and lower fasting ghrelin, with a blunted ghrelin response to the drink and higher appetite at 60 min. In addition, the insulin and PYY responses were significantly greater in males compared to females. When pubertal stage was examined in obese males (Experiment 2), PYY increases were higher, and ghrelin lower, in mid-late compared to pre-early puberty.

Higher fasting and postprandial insulin in obese adolescents is well documented and related to reduced hepatic and peripheral insulin sensitivity as was observed in our participants(45, 254). Of note, males had similar fasting, but higher postprandial insulin compared to females, with the most pronounced response occurring in obese males, even after adjusting for fat mass. In contrast, studies report higher insulin AUC after an OGTT in females (mean age = ~13 y)(129) and greater insulin resistance, as measured by HOMA-IR, in females (12-19 y) compared to males after accounting for age, weight and race (263). It is possible that our findings may relate to sex-dependent differences in insulin response to protein ingestion compared to glucose alone; however, further study is needed to evaluate this finding. Similarly, another study reported higher fasting insulin levels in obese pubertal (Tanner II-V) compared to obese prepubertal (Tanner I) children, with no comparison between sexes (43). We found no difference in insulin concentrations between pre-early pubertal and mid-late pubertal obese boys. Although pubertal insulin resistance is a physiological event (147) attributable to increased growth hormone and IGF-1 with advancing puberty (150), fat mass is independently associated with insulin resistance, and the extreme adiposity in our population may have obscured any changes in insulin attributable to different stages of puberty.
In our study, PYY was positively associated with body weight and was higher in obese adolescents, especially males. In adults fasting PYY_{3-36} is positively related to obesity-associated insulin resistance and type 2 diabetes (264). In children, one previous study showed a trend for higher total PYY in overweight compared to NW children (7 – 10 y) and a positive correlation between fasting PYY and percent body fat (54). PYY secretion has been shown to be greater after consumption of a higher protein (36% carbohydrate, 44% protein, 20% fat) meal in obese children (45). These findings are in accordance with ours using a glucose and WP drink. Lastly, a weight reduction study in children(88) demonstrated significant increases in PYY one year after intervention with BMI reduction of 0.67 kg/m^2. It remains unclear as to whether higher PYY is a response to increasing adiposity and reduced energy needs of the participants or similar to insulin, a central PYY resistance develops leading to blunted satiety in human obesity(54, 264). No studies have compared male versus female postprandial levels of PYY, and our findings are unique, but require further validation.

Fasting and postprandial ghrelin are lower in obese compared to NW adolescents and are related to insulin sensitivity (76), consistent with previous findings (43-47). It has been postulated (78) that the rise in postprandial insulin activates a short-term feedback loop resulting in a decrease in ghrelin; however, increasing adiposity delays this drop in ghrelin. Blunted ghrelin, as observed in the current study, may delay satiation and increase FI in obese adolescents.

Finally, we found that stage of puberty in obese boys was related to PYY and ghrelin response. Lower fasting PYY in the mid-late pubertal obese males is similar to a previous study that reported lower fasting PYY in mid-late pubertal adolescents (Tanner III-IV) when growth hormone was highest, as a potential mechanism to increase FI needed for growth (129).
However, insulin sensitivity may also mediate postprandial PYY concentrations. The percent change in PYY was higher in the mid-late pubertal than pre-early pubertal obese males and this change in PYY (0-30min) was negatively associated with WBISI ($r = -0.954$, $p < 0.001$). In line with reports that ghrelin levels decline with advancing puberty (43, 140), mid-late pubertal obese males had lower fasting ghrelin concentrations, as previously shown in a study of pubertal versus prepubertal children (43). Lower ghrelin in mid-late pubertal males has been shown to be inversely related to testosterone, which may account for these findings (142).

Appetite hormone responses in obese and MLP obese adolescents did not explain the higher appetite 60 min post-drink. In obesity, it appears that acute appetite hormone levels and subjective appetite are disconnected, and are in line with the appetite hormone resistance (higher insulin and PYY, blunted ghrelin) observed in this study. Higher appetite in MLP obese males is in accordance with the greater energy needs in later puberty (116) and its relationship with FI requires further investigation.

Although our study is the first to describe the effect of obesity, sex and pubertal status on appetite hormone responses after a mixed beverage, there are a few limitations worth noting. First, our sample size in Experiment 1 of males and females within each weight group was small ($n = 4-5$). Second, since our study is exploratory in nature, we did not measure FI and were not able to relate FI with appetite hormone responses. Third, in Experiment 2, only obese males were studied, and future studies examining pubertal status should include NW and obese female participants.

4.6 CONCLUSION
In conclusion, obesity, sex and pubertal status affect macronutrient-stimulated appetite hormone secretion. How these factors contribute to short and long term regulation of FI requires further investigation in children during this rapid period of growth and development.
5 Pubertal status affects children’s food intake response to carbohydrate, but not protein drinks

5.1 ABSTRACT

**Background:** The effect of puberty on food intake after pre-meal glucose and whey protein drinks was determined in 9 – 14 y old children (5th – 99th percentile).

**Methods:** On 6 separate mornings, boys (Experiment 1, n=14 pre-early pubertal, 15 mid-late pubertal) and girls (Experiment 2, n=13 pre-early pubertal, 16 mid-late pubertal) randomly received equally sweetened drinks containing Sucralose® (control), glucose or whey protein (0.75 g/kg body weight) in 250 mL of water 2 h after a standardized breakfast. *Ad libitum* food intake was measured either 30 or 60 min later and appetite was measured over time.

**Results:** Puberty affected food intake differently in boys and girls and was macronutrient dependent. Mid-late compared to pre-early pubertal boys had greater food intake (p=0.003), but not when food intake was corrected for body weight (p=0.589). Conversely, food intake/kg body weight was lower in mid-late than pre-early pubertal girls (p=0.024). In mid-late pubertal children, mealtime compensation for energy from glucose was less at 60 than at 30 min (p<0.017), but not for whey. However, compensation for either drink was not different at 30 and 60 min meals in pre-early pubertal children (p>0.05). In all children, glucose and whey suppressed food intake similarly compared to control at 30 min, but only whey suppressed food intake at 60 min (p<0.05). Appetite was associated with food intake in mid-late pubertal children only.

**Conclusions:** Pubertal status affects children’s response to carbohydrate, but not protein drinks.
5.2 INTRODUCTION

Puberty is a complex and coordinated process involving significant physical maturational changes from childhood to adulthood, including rapid linear growth and changes in proportion of fat and fat free mass (108). Changes in body composition occur alongside alterations in energy intake (114). Children’s food intake (FI) patterns are concordant with sex-specific changes in body composition and peak growth velocity to match changing energy requirements during pubertal development: energy intake increases with pubertal development (116). As well, environmental factors at mealtime affecting FI are influenced by puberty. For example, television viewing (TVV) at mealtime reduced caloric compensation after consumption of a glucose (g/kg body weight) drink in peripubertal, but not postpubertal, girls (257).

Protein contributes strongly to short-term satiety and reduces FI more than carbohydrates in adults (236). Only one study has examined the effect of protein and carbohydrate on FI in children. Both normal weight (NW) and obese boys (9 – 14 y) reduced their FI 30 min after consumption of a fixed amount of glucose (50 g) compared to a non-caloric control drink (228). Although the same amount of whey protein (WP, 50 g) reduced FI similar to glucose in the NW boys, it had no effect in the obese boys. Furthermore, WP given relative to body weight on a g/kg basis, resulted in the lowest FI 60 min after consumption compared to glucose in NW boys (228). However, appetite and FI response to protein and carbohydrate beverages has not been reported in children during the development of puberty. Thus, the hypothesis of this study was that puberty affects compensation for energy intake in beverages. Therefore, our objective was to determine the effect of pubertal status on subjective appetite and FI following consumption of
glucose and WP drinks. Participants included male (Experiment 1) and female (Experiment 2) 9 – 14 y old children (5 – 99th percentile) at Tanner stages I-V of puberty.

5.3 MATERIALS AND METHODS

5.3.1 Study Design

Two experiments were conducted using a single-blinded, within-subject, repeated measures design. Children were randomly assigned to a counterbalanced treatment order, using a randomized block design, which was generated with a random generator script in SAS version 9.2. Participants were blinded about the type of treatment ingested. On six separate mornings, 1 week apart, each of the pre-early and mid-late pubertal boys (Experiment 1) and girls (Experiment 2) were given equally sweetened preloads of SPLENDA Sucralose (control), glucose or WP made up to 250 mL with water 2 h after a standardized breakfast. Thirty or 60 min after drink consumption, FI was measured at an ad libitum pizza meal.

5.3.2 Subjects

Nine to fourteen year old children were recruited through newspaper advertisements and by word-of-mouth. Children born at full-term and of normal birth weight were eligible. Those not consuming breakfast regularly, dieting, taking medication, unable to follow study instructions and disliking the treatment or foods offered for breakfast or lunch were excluded. A screening was scheduled for the eligible child and parent at the Department of Nutritional Sciences, University of Toronto, where informed written consent was obtained from the parent and written assent from the child. Height (m) and weight (kg) were measured, and age-and-sex
specific BMI percentiles were calculated according to the WHO growth charts. (9) Bioelectrical impedance analysis was used to estimate body composition (RJL Systems BIA, 101Q) based on the Horlick equation (259). Pubertal status was self-assessed using gender-appropriate Tanner drawings (encompassing the 5 stages of puberty) (265, 266), which are accurate in reporting approximate stages of pubertal development (267). Children in Tanner stages I-II were categorized as pre-early pubertal, while children in Tanner stages III-V were categorized as mid-late pubertal. Children were informed that they should refrain from moderate-vigorous activity 10 h prior to the study session. The Dutch Eating Behaviour Questionnaire was administered to all children to assess restrained eating (scores ranged from 1.5 – 1.7) (268). This study was conducted according to guidelines laid down in the Declaration of Helsinki and all procedures were approved by The Human Subjects Review Committee, University of Toronto Canada (Ref #21595).

5.3.3 Experimental Procedure

Procedures are similar to those reported previously (228, 257). On six separate mornings, 7 d apart, children arrived at the Department between 0900 and 1200, 2 h after consuming a standardized breakfast of Parmalat® fat-free skim milk (250 ml, 91 kcal), Honey Nut Cheerios® (26 g, 103 kcal donated by General Mills, Inc.) and Tropicana Orange Juice® (236 ml, 110 kcal) at home. Children were interviewed to ensure their compliance and those who deviated from the protocol were rescheduled. Appetite visual analogue scales (VAS) were administered at baseline (0 min), at regular intervals (15-30 min) prior to mealtime and immediately post-meal. Sweetness and palatability VAS were assessed by “How pleasant have you found the drink/pizza? (“not pleasant at all” to “very pleasant”) and were administered immediately after
drink consumption or post-meal. Motivation-to-eat VAS scale, which measure dimensions of subjective appetite (269), was composed of four 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 full do you feel? (“not full at all” to “very full”), and (4) How much food do you think you can eat? (prospective food consumption) (“nothing at all” to “a large amount”). Children were instructed to read each question and place an “x” along the 100 mm line depending on their current feelings.

Three equally sweetened drinks were administered. Providing glucose and WP relative to body weight was predicted to reduce the variability in response occurring from differences in body fatness among children. Thus, two drinks contained either 0.75 g/kg body weight glucose monohydrate (Grain Process Enterprises, Toronto, ON Canada) or WP isolate (chocolate whey-protein isolate; Interactive Nutrition International Inc., Ottawa, Ontario, Canada), an amount that was palatable for the larger children to consume. The third drink acted as a control, matched for sweetness and flavor by addition of the high-intensity sweetener SPLENDA® Sucralose (donated by Tate and Lyle Sucralose, Inc. Deautur, IL), since it is not metabolized in the body and does not alter blood glucose or insulin secretion (270). Aspartame-sweetened, orange-flavored crystals (1.1 g, Sugar Free Kool-Aid, Kraft Canada Inc., Don Mills, ON Canada) were added to the glucose and control drink to standardize flavor. Chocolate WP was selected as it was more palatable for the children as determined by a taste panel conducted prior to the study. Drinks were prepared 1 day prior to consumption in covered, opaque cups and stored in the refrigerator. The following morning, subjects consumed the chilled drinks, followed by 50 mL of water to minimize aftertaste. After drink consumption, children engaged in quiet entertainment (cards, board games, etc.).
Thirty or 60 min after drink consumption and after rating their appetite, participants were escorted to a feeding room with individual cubicles and served an *ad libitum* pizza lunch along with a 500 mL glass of filtered water for 30 min. Children were asked to remain seated for the 30 min duration and were instructed to eat until “comfortably full.” A freshly baked tray of pizza was provided every 10 min starting 30 or 60 min after drink consumption. Two varieties of Deep ‘N Delicious 5” diameter pizza (~ 200 kcal each) were used; pepperoni and three-cheese pizzas (McCain Foods Ltd, Florenceville, NB, Canada). Each tray contained 3 pizzas: 2 of their first choice and 1 of their second choice (baked 8 min at 430°F). Cooked pizzas were weighed and cut into four equal pieces before serving, and the amount left after the meal was subtracted from the initial weight to measure FI. Each variety of pizza was weighed separately and energy consumed (in kcal) was calculated using manufacturer information. Water was weighed before and after the meal to calculate the net amount ingested. Post-meal, children rated their appetite and meal palatability.

5.3.4 Statistical analyses

Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc., Carey, NC) was used for statistical analyses. Two-tailed tests were used to determine differences in baseline subject characteristics between pubertal groups in each experiment. Body weight was initially included as a potential factor affecting FI and appetite, but because there were no significant effects, it was subsequently removed from the model. However, because of the large weight differences between pre-early vs. mid-late pubertal children, FI in kcal was also corrected for body weight (kg). Within each experiment, a 3-way repeated measures analysis of variance (ANOVA) using the Proc Mixed procedure was used to analyze the effects of pubertal status (pre-early vs. mid-
late), time (30 vs. 60 min), drink (control vs. glucose vs. WP) and their interactions on food and water intake in each experiment. When drinks were given 30 or 60 min pre-meal, subjective appetite (reported as the change from baseline) was separated into pre-meal (0 – 30 min or 0 – 60 min) and post-meal (30 – 60 min or 60 – 90 min) periods. Within the pre-early and mid-late pubertal males and females, the pre-meal period was assessed using a 2-way repeated measures ANOVA with time and drink as factors; the post-meal period, sweetness of the drink and pleasantness of the drink/pizza meal was assessed using a one-way repeated measures ANOVA with drink as the main factor. Caloric compensation, which is the amount of food compensated for after each caloric drink, was assessed using a paired two-tailed t-test when the meal was given at 30 and 60 min for each treatment. Post-hoc analysis by the Tukey-Kramer test was performed when treatment effects were found to be statistically significant.

An average appetite score was calculated at each time of measurement for each drink treatment by the formula: Appetite score = [desire to eat + hunger + (100 – fullness) + prospective food consumption]/4, which reflects the 4 questions on the motivation-to-eat VAS as used previously (257, 271). FI was determined from the total energy content of the pizza consumed at the test meal. Cumulative energy intake was calculated from the sum of calories from the drink and meal. Percent caloric compensation was calculated for each person by the following formula:(272) Compensation (%) = [Control intake (kcal) – Treatment intake (kcal)/kcal in treatment drink] x 100. Data are presented as means ± standard error of mean (SEM). Significance was considered at p < 0.05. Correlations on dependent measures were conducted by use of Pearson correlation coefficients.
Based on our earlier studies in children (228, 257), a 123 kcal decrease in meal-time consumption with a sample size of 29 (13-16/group) is powered (> 80%) to see the effect of a caloric beverage compared with the control with a within-subject SD of 232 kcal.

**5.4 RESULTS**

5.4.1 Subjects

In Experiment 1 (n = 29, **Table 5.1**), thirteen boys were between the 21\textsuperscript{st} and 85\textsuperscript{th} (NW), eleven were between the 85\textsuperscript{th} and 97\textsuperscript{th} (overweight), and five were greater than the 97\textsuperscript{th} (obese) age- and sex- specific BMI percentiles (9). In Experiment 2, (n = 29, **Table 5.1**), sixteen girls were between the 5\textsuperscript{th} and 85\textsuperscript{th} (NW), eight were between the 85\textsuperscript{th} and 95\textsuperscript{th} (overweight), and five were greater than the 97\textsuperscript{th} (obese) age- and sex- specific BMI percentiles (9). There were two dropouts due to non-compliance.

**Table 5.1: Baseline characteristics of test subjects\textsuperscript{1}**

<table>
<thead>
<tr>
<th></th>
<th>Early Pubertal (n=14)</th>
<th>Mid-late Pubertal (n=15)</th>
<th>Early Pubertal (n=13)</th>
<th>Mid-late Pubertal (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>10.3 ± 0.2\textsuperscript{*}</td>
<td>12.8 ± 0.4</td>
<td>10.2 ± 0.2\textsuperscript{*}</td>
<td>13.1 ± 0.3</td>
</tr>
<tr>
<td><strong>NW:OW/OB</strong></td>
<td>6:8</td>
<td>7:8</td>
<td>8:5</td>
<td>8:8</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>40.9 ± 2.1\textsuperscript{*}</td>
<td>61.0 ± 4.3</td>
<td>42.3 ± 3.8\textsuperscript{*}</td>
<td>58.7 ± 2.5</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>143.7 ± 2.2\textsuperscript{*}</td>
<td>161.0 ± 2.2</td>
<td>142.7 ± 2.7\textsuperscript{*}</td>
<td>161.2 ± 2.1</td>
</tr>
<tr>
<td><strong>BMI percentile</strong></td>
<td>78.1 ± 6.7</td>
<td>73.3 ± 7.3</td>
<td>68.8 ± 9.1</td>
<td>73.8 ± 6.3</td>
</tr>
<tr>
<td><strong>Fat mass\textsuperscript{2} (kg)</strong></td>
<td>9.8 ± 1.1</td>
<td>15.6 ± 3.1</td>
<td>14.5 ± 2.6</td>
<td>19.1 ± 2.2</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>30.0 ± 1.0*</td>
<td>46.7 ± 2.9</td>
<td>28.7 ± 1.8*</td>
<td>39.6 ± 1.1</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Restrained eating</strong>³</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

¹Data are presented as means ± SEM. *Significantly different from mid-late pubertal p < 0.001 within each experiment, unpaired t-test.

²Body composition determined from Bioelectrical Impedance Analysis based on the Horlick equation.

³Restrained eating was measured with the Dutch Eating Behaviour Questionnaire(268).

5.4.2 Energy and water intake

**Experiment 1 – Boys:** Unadjusted FI (kcal, Table 5.2) was affected by pubertal status (p = 0.003), drink (p < 0.001), but not time-to-the-meal (p = 0.133). Mid-late compared to pre-early pubertal boys (p = 0.003) had higher FI. Glucose reduced FI compared to control (7%, p = 0.028) and WP reduced FI compared to glucose (10%, p = 0.004) and control (16%, p < 0.001). A drink-by-time-to-the-meal interaction (p < 0.003, data not shown) showed that glucose and WP drinks reduced FI at 30 min compared to control (p < 0.002), but only WP reduced FI at 60 min (p < 0.022) compared to control and glucose.
When FI was corrected for body weight, FI/kg body weight and cumulative energy intake/kg body weight were affected by drink (p < 0.007, Table 5.2), but not pubertal status or time-to-the-next meal (p > 0.050). Glucose reduced FI/kg body weight (8%, p = 0.020) and WP compared to glucose (10%, p = 0.001) and compared to control (17%, p < 0.001) in all boys. A drink-by-time-to-the-meal interaction (p = 0.001, Figure 5.1A) showed that glucose and WP drinks reduced FI/kg body weight at 30 min compared to control (~18%, p < 0.001), but only WP reduced FI/kg body weight at 60 min (18%, p < 0.010). Glucose resulted in an increased cumulative energy intake/kg body weight compared to control and WP (~8%, p < 0.019). A drink-by-time-to-the-meal interaction (p = 0.002, Figure 5.1B) showed this to be due to an increase in cumulative energy intake at 60 min after the glucose drink, compared to control and WP (16%, p = 0.001), and no differences at 30 min. Water intake (g/kg body weight) was not affected by pubertal status, drink or time-to-the-meal (p > 0.05, Table 5.2).

Table 5.2: Experiment 1: Effect of pubertal status, drink and time-to-the-meal on FI, cumulative energy intake and water intake in boys

<table>
<thead>
<tr>
<th>Pubertal status</th>
<th>Drink</th>
<th>Time-to-the-meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Mid-late</td>
</tr>
<tr>
<td>Food intake² (kcal)</td>
<td>724 ± 27</td>
<td>1019 ± 33²</td>
</tr>
<tr>
<td>Food intake³ (kcal/kg BW)</td>
<td>17.8 ± 0.5</td>
<td>16.9 ± 0.5</td>
</tr>
</tbody>
</table>
Data are means ± SEM; (n = 29). A 3-factor ANOVA for food intake and cumulative energy intake was used with pubertal status, drink, and time-to-the-meal as main factors. Different superscripts are significantly different (p < 0.05).

Food intake was affected by pubertal status (p = 0.003), drink (p < 0.001) and a drink-by-time-to-the-meal interaction (p < 0.001), but not time-to-the-meal (p = 0.133). All other interactions were p > 0.05.

Food intake per kilogram body weight was affected by drink (p < 0.001) and a drink-by-time-to-the-meal interaction (p < 0.001), but not pubertal status (p = 0.589) or time-to-the-meal (p = 0.256). All other interactions were p > 0.05.

Cumulative energy intake per kilogram body weight (drink + pizza) was affected by drink (p < 0.001), but not pubertal status (p = 0.568) or time-to-the-meal (p = 0.259), and a drink-by-time-to-the-meal interaction (p < 0.001). All other interactions were p > 0.05.

Water consumed at the test meal was not affected by pubertal status drink or time-to-the-meal p > 0.05.

<table>
<thead>
<tr>
<th>Cumulative energy intake (kcal/kg BW)</th>
<th>19.9 ± 0.5</th>
<th>19.1 ± 0.5</th>
<th>18.9 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</th>
<th>20.6 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</th>
<th>19.1 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</th>
<th>19.9 ± 0.5</th>
<th>19.1 ± 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (g/kg BW)</td>
<td>5.6 ± 0.4</td>
<td>5.1 ± 0.3</td>
<td>5.0 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>5.4 ± 0.3</td>
<td>5.3 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are means ± SEM; (n = 29). A 3-factor ANOVA for food intake and cumulative energy intake was used with pubertal status, drink, and time-to-the-meal as main factors. Different superscripts are significantly different (p < 0.05).

<sup>2</sup>Food intake was affected by pubertal status (p = 0.003), drink (p < 0.001) and a drink-by-time-to-the-meal interaction (p < 0.001), but not time-to-the-meal (p = 0.133). All other interactions were p > 0.05.

<sup>3</sup>Food intake per kilogram body weight was affected by drink (p < 0.001) and a drink-by-time-to-the-meal interaction (p < 0.001), but not pubertal status (p = 0.589) or time-to-the-meal (p = 0.256). All other interactions were p > 0.05.

<sup>4</sup>Cumulative energy intake per kilogram body weight (drink + pizza) was affected by drink (p < 0.001), but not pubertal status (p = 0.568) or time-to-the-meal (p = 0.259), and a drink-by-time-to-the-meal interaction (p < 0.001). All other interactions were p > 0.05.

<sup>5</sup>Water consumed at the test meal was not affected by pubertal status drink or time-to-the-meal p > 0.05.
Figure 5.1: Experiment 1: Interaction between drink composition and time-to-the-meal in all boys for a) Food intake and b) Cumulative energy intake. Bars with different superscripts are significantly different (Tukey-Kramer post hoc test, p < 0.05). Values are mean ± SEM (n = 29 boys)
Experiment 2 – Girls: Unadjusted FI (kcal, Table 5.3) was not affected by pubertal status or time-to-the-next meal (kcal, p > 0.050). However, there was an effect of drink (p < 0.001), whereby glucose and WP reduced FI similarly by 18% compared to control (p < 0.001). A drink-by-time-to-the-meal interaction (p = 0.028, data not shown) showed that glucose and WP drinks reduced FI at 30 min compared to control (p < 0.001), but only WP reduced FI at 60 min (p = 0.022) compared to control.

An effect of pubertal status showed that FI/kg body weight was ~29% and cumulative energy intake/kg body weight was ~26% lower in mid-late compared to pre-early pubertal girls (p < 0.027, Table 5.3). Drink (p < 0.001), but not time-to-the-meal affected FI/kg body weight at the test meal (Table 5.3). Glucose and WP reduced FI/kg body weight similarly by ~19% compared to control (p < 0.001). Cumulative energy intake/kg body weight was not affected by drink or time-to-the-meal (p > 0.05, Table 5.3). Water intake (g/kg body weight) was affected by drink (p = 0.025), but not pubertal status or time-to-the-meal (p > 0.05, Table 5.3). There were no significant interactions for FI/kg body weight, cumulative energy intake/kg body weight or water intake (p > 0.05).
Table 5.3: Effect of pubertal status, drink and time-to-the-meal on FI, cumulative energy intake and water intake in girls

<table>
<thead>
<tr>
<th>Pubertal status</th>
<th>Drink</th>
<th>Time-to-the-meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Mid-late</td>
</tr>
<tr>
<td>Food intake(^2) (kcal)</td>
<td>717 ± 32</td>
<td>693 ± 25</td>
</tr>
<tr>
<td>Food intake(^3) (kcal/kg BW)</td>
<td>17.0 ± 0.7(^a)</td>
<td>12.0 ± 0.4(^a)</td>
</tr>
<tr>
<td>Cumulative energy intake(^4) (kcal/kg BW)</td>
<td>19.1 ± 0.7(^a)</td>
<td>14.3 ± 0.4(^b)</td>
</tr>
<tr>
<td>Water intake(^5) (g/kg BW)</td>
<td>5.2 ± 0.4</td>
<td>4.3 ± 0.3</td>
</tr>
</tbody>
</table>

\(^1\) Data are means ± SEM; (n = 29). A 3-factor ANOVA for food intake and cumulative energy intake was used with pubertal status, drink, and time-to-the-meal as main factors. Different superscripts are significantly different (p < 0.05).

\(^2\) Food intake was affected by drink (p < 0.001), but not pubertal status (p = 0.718) or time-to-the-meal (p = 0.620) and a drink-by-time-to-the-meal interaction (p = 0.028). All other interactions were p > 0.05.

\(^3\) Food intake per kilogram body weight was affected by pubertal status (p = 0.024) and drink (p < 0.001), but not time-to-the-meal (p = 0.720). All interactions were p > 0.05.

\(^4\) Cumulative energy intake per kilogram body weight (drink + pizza) was affected by pubertal status (p = 0.026), but not drink (p = 0.102) or time-to-the-meal (p = 0.577). All interactions were p > 0.05.
Water consumed at the test meal was affected by drink (p = 0.025), not pubertal status (p = 0.337) or time-to-the-meal (p = 0.411). All interactions were p > 0.05.

5.4.3 Caloric compensation

Experiment 1 – Boys: In pre-early pubertal boys, compensation for glucose (70%) and WP (128%) at 30 min did not differ from glucose (19%, p = 0.161) and WP (123%, p = 0.834) at 60 min. In mid-late pubertal boys, compensation for WP (75%) at 30 min did not differ from 60 min (94%, p = 0.904); however, compensation for glucose (112 vs. -21%, p = 0.001, Figure 5.2a) decreased over time.

Experiment 2 – Girls: In pre-early pubertal girls, compensation for glucose (73%) and WP (90%) at 30 min did not differ from glucose (52%, p = 0.713) and whey (116%, p = 0.522) at 60 min. In mid-late pubertal girls, compensation for WP (75%) at 30 min did not differ from 60 min (50%, p = 0.256); however, compensation for glucose decreased over time in mid-late pubertal girls only (122 vs. 40%, p = 0.016, Figure 5.2b).
**Figure 5.2:** Caloric compensation (□: glucose ■: whey) in pre-early pubertal a) boys and b) girls and in mid-late pubertal c) boys and d) girls at 30 and 60 min after drink consumption.

*significantly different from glucose at 60 min. (paired t-test, p < 0.05). Values are mean ± SEM
(Boys: n = 14; pre-early pubertal, n = 15; mid-late pubertal. Girls: n = 13; pre-early pubertal, n = 16; mid-late pubertal).

5.4.4 Pre-meal (0 – 30 min) and post-meal (60 min) minus pre-meal appetite scores

In both pre-early and mid-late pubertal boys (Experiment 1, Appendix 6 Table 5.4) and girls (Experiment 2, Appendix 6 Table 5.5), pre-meal (0 – 30 min) average appetite scores increased over time (p < 0.05) when the test meal was at 30 min, but were not affected by drink or a drink-by-time interaction. In pre-early and mid-late pubertal boys (Experiment 1), post-meal (60 min) minus pre-meal (30 min) average appetite scores were reduced by the meal. Drink affected post-meal average appetite (p = 0.028, data not shown), with WP reducing scores (-69 ± 5.5) more than glucose (-47 ± 7.6, p = 0.048) in pre-early pubertal boys only. In pre-early and mid-late pubertal girls (Experiment 2), post-meal (60 min) minus pre-meal (30 min) average appetite scores were reduced by the meal, but were not affected by drink or a drink-by-time interaction (data not shown).

5.4.5 Pre-meal (0 – 60 min) and post-meal (90 min) minus pre-meal appetite scores

In both pre-early and mid-late pubertal boys (Experiment 1, Appendix 6 Table 5.4) and girls (Experiment 2, Appendix 6 Table 5.5), pre-meal (0 – 60 min) average appetite increased over time (p < 0.05) when the test meal was at 60 min. Pre-early pubertal boys (Experiment 1) had lower average appetite after glucose than control (p = 0.010), whereas mid-late pubertal boys had higher average appetite after glucose compared to control (p = 0.039). In mid-late girls only
(Experiment 2), average appetite scores were higher after control compared to glucose and WP (p < 0.004). In both pre-early and mid-late pubertal boys (Experiment 1) and girls (Experiment 2), post-meal (90 min) minus pre-meal (60 min) average appetite scores were reduced by the meal, but were not affected by drink in either group (data not shown).

5.4.6 Sweetness and pleasantness of the drinks and pleasantness of the test meal

Overall, there was no significant effect of drink on pleasantness of the meal for either boys or girls. Drink composition affected sweetness in pre-early pubertal boys only (p = 0.047), with lower scores for WP than control (p = 0.047). Drink composition affected pleasantness in pre-early and mid-late pubertal boys and girls (p < 0.008), with lower scores for WP than glucose and control (p < 0.032, Appendix 6 Table 5.6).

5.4.7 Associations between average appetite and food intake

**Experiment 1 – Boys:** In pre-early pubertal boys, average appetite (r = -0.188, p = 0.519) was not associated with FI at 30 min and similar results were observed at 60 min. In mid-late pubertal boys, average appetite (r = 0.591, p = 0.020) was positively associated with FI at 30, but not 60, min.

**Experiment 2 – Girls:** In pre-early pubertal girls, there was no association between average appetite (r = 0.209, p = 0.494) with FI at 30 min and similar results were observed at 60 min. In mid-late pubertal girls, average appetite (r = 0.770, p = 0.001) was positively associated with FI at 30, but not 60, min.
5.5 DISCUSSION

This is the first study to examine caloric compensation to pre-meal drinks during pubertal development in both boys and girls. These results support our hypothesis that FI compensation in a beverage is affected by puberty and its macronutrient composition; however, pubertal status affected FI differently in boys and girls and was macronutrient dependent.

Pubertal status was clearly a factor affecting FI, but the effect was different in boys and girls and for glucose and protein drinks. Mid-late pubertal boys had greater FI (kcal) compared to pre-early pubertal boys; however, when FI was corrected for body weight, mid-late pubertal girls had lower FI/kg body weight compared to pre-early pubertal girls. A recent study reported that greater FI with increasing pubertal development in males and females was explained by fat mass, fat free mass and body weight, with little effect of puberty (116). Our results in the boys but not girls are consistent with these previous findings, suggesting that FI during pubertal development is largely a reflection of changes in body composition, with greater accumulation of fat free mass in males and greater increases in fat mass in females (273). However, the age range of our children was narrower (9-14 vs. 8-17 y) and classification of pubertal stages was different in the current study: (pre-early puberty [Tanner I-II] and mid-late puberty [Tanner III-V] vs. prepuberty, [Tanner I]; early-mid puberty [Tanner II-III] and late puberty [Tanner IV-V]. These differences may explain why FI/kg body weight was greater in pre-early pubertal girls. Furthermore, early-mid puberty is a period of rapid growth for females (112), while those in later puberty experience hormonal changes that inhibit FI (274).

Mid-late, but not pre-early, pubertal boys and girls compensated fully at 30 min for the energy in the glucose drink. This is consistent with previous reports demonstrating that children
compensate in later meals for sugars consumed as liquids, suggesting that sugars do not bypass physiologic regulatory systems (272, 275). Partial compensation in the pre-early pubertal boys and girls was consistent with another study in children of the same age range (9-10 y), whose compensation at 30 min for a pre-meal sucrose (45 g) drink was 68% (276). However, there may be several explanations for why compensation decreased after glucose from 30 to 60 min in the mid-late, but not pre-early, pubertal boys and girls. First, age may be a factor: younger children (6 – 7 y) had greater compensation for the energy content of low energy (783 kJ) and high energy (1628 kJ) pre-meal snacks given 90 min before a test meal compared to older children (8 – 9 y) (207). Furthermore, mid-late pubertal children are more insulin resistant than pre-early pubertal children which mediates appetite hormones, such as orexigenic ghrelin. Ghrelin was suppressed after an oral glucose load at 60 min in prepubertal, but not early pubertal boys (143). This suggests that children in later puberty have impaired appetite hormone regulation to carbohydrates that may have implications for weight reduction recommendations.

In contrast, compensation in both pre-early and mid-late pubertal children for the WP beverage was sustained over time. However, mid-late compared to pre-early pubertal girls had lower compensation for the WP drink at 60 min (50 vs. 116%, respectively), in contrast to NW boys who fully compensated (228), suggesting a possible contribution of sex hormones. During puberty, estrogen affects FI regulation and the action of appetite hormones including ghrelin and cholecystokinin (126). Estrogen fluctuations during the follicular and luteal phases of the menstrual cycle (121) may have contributed to the lack of compensation to the protein drink.

Composition of the pre-meal drink was a factor: glucose and WP reduced overall FI compared to control, with WP resulting in the greatest reduction in boys. Glucose was compared with WP because studies in adults demonstrate that proteins suppress short-term FI more than
carbohydrate or fat as indicated by both quantitative and subjective measures (209). Furthermore, NW and obese boys respond differently to WP, with dose a factor in the response (228). The FI differences between WP and glucose correspond to their postingestive effects. Whey requires digestion before absorption, resulting in a rapid and sustained increase in plasma amino acids (235), and provides a slower stimulation of satiety hormones cholecystokinin and glucagon-like peptide-1 (235). Conversely, glucose in solution is rapidly absorbed to stimulate satiety hormones glucagon-like peptide-1 and insulin (277). Our results are the first to show that glucose and WP similarly reduced overall FI in the girls compared to control, suggesting that sex differences in energy intake regulation exist in adolescence. Similar to NW men (236), WP did not increase cumulative energy intake in either boys or girls; thus pre-meal consumption of WP may be used to prevent excess intake during later meals in children.

In contrast to the decrease in FI after the caloric drinks, appetite did not decrease in either pre-early or mid-late pubertal children, which is consistent with other reports in children (228, 257). Pre-meal average appetite over 60 min was greater after the control drink in the pre-early pubertal boys and mid-late pubertal girls, whereas lower average appetite (increased satiety) was found after caloric ad libitum solid snacks in NW children (8-11 y) compared to water (278). These results suggest that subjective appetite is more accurately interpreted after solids than liquids. Similarly, children consistently report reductions in appetite and increased fullness after the test meal (228, 257), and the pre-early pubertal boys reported reduced appetite after the WP drink. Because measures of appetite were positively associated with FI at 30 min in mid-late pubertal boys and girls only, it can be suggested that subjective appetite is a learned association with FI and that children in pre-early puberty are yet unable to make this association (181), consistent with a previous report (257). However, the lack of association between appetite and FI
at 60 min in mid-late pubertal children suggests that they may be less sensitive to predicting their intake over longer time periods.

Our study has limitations that are worth noting. First, our children were self-staged using gender-appropriate Tanner drawings (265, 266), and may have under or overestimated their pubertal growth. However, physical examination of the children by a physician trained in Tanner Staging was considered too invasive for this type of study conducted in healthy children from the community. Second, although we did not account for menstrual cycle phase, we were not powered to evaluate the effects of FI and phase with the current study design. Third, due to the short-term nature of this study, the results cannot be generalized to longer-term studies or overall daily caloric intake.

5.6 CONCLUSION

In conclusion, pubertal status affects children’s response to carbohydrate, but not protein drinks. Therefore, recommendations for managing FI during puberty should consider the macronutrient content of the diet. In preference to glucose, WP can be used as a pre-meal beverage to maximize short-term satiety in children and adolescents. These findings should be evaluated further in longitudinal studies, and may lead to directed strategies for obesity management in this age group.
Television viewing at mealtime reduces caloric compensation in peripubertal, but not postpubertal, girls

6.1 ABSTRACT

The effect of TV viewing (TVV) and pubertal status of 9 – 14 y old girls on mealtime food intake (FI) after a pre-meal glucose drink was determined. On 4 separate mornings, girls randomly received equally sweetened drinks containing Sucralose® (control) or glucose (1.0 g/kg body weight) in 250 mL of water 2 h after a standardized breakfast. FI from an ad libitum pizza meal was measured 30 min later with or without TVV. Appetite was measured at 15 min intervals to lunch and post-meal. TVV at mealtime had no effect on FI, however, glucose suppressed FI more with no TVV compared with TVV (24% vs. 10%, p < 0.001), primarily due to its effect in post-pubertal girls (p < 0.028). In post-pubertal girls (n = 8), glucose reduced FI by ~27% in both the no TVV and TVV conditions, but in peri-pubertal girls (n = 17), reduction in FI was 22% without TVV and only 1% while TVV. Appetite correlated with FI at 30 min only in post-pubertal girls. TVV at mealtime reduced caloric compensation following consumption of the glucose drink in peri-pubertal, but not post-pubertal, girls, with no effect on mealtime FI.

(Clinical trial number NCT01025687).

This work has been published in Pediatric Research (2011) 70(5):513-7.
6.2 INTRODUCTION

Food intake (FI) regulation in children is influenced by both physiological and environmental factors. Physiological signals of satiety and satiation are primary regulators of FI and energy balance (279). However, many non-food related stimuli in the mealtime environment impact this regulation (200). In children, excessive screen time (4+ h/d) has been associated with increased incidence of obesity (185). Possible causes of this association are increased preference for energy dense foods and sweetened beverages (188), decreased resting metabolic rate (189), meal skipping (190) and reduced activity (280) while television viewing (TVV).

Despite the strong associations found between obesity and TVV (280), few studies in children and adolescents have reported quantitative FI while TVV using a within-subject design. While 3 – 5 y olds eat less while TVV (203), 15 – 16 y olds show no differences in FI when eating while TVV, no TVV and listening to music (204). Only one study examined the effect of TVV on FI in 9 – 14 y old children (206). While TVV, boys ate 24% more at a pizza meal, indicating delayed satiation and showed diminished response to satiety signals following a glucose drink taken 30 min before the meal. Thus, mealtime TVV impaired physiologic signals leading to both satiety and satiation (206).

The effect of TVV on satiety and satiation has not been reported for 9 – 14 y old girls. Girls in this age range experience hormonal changes during puberty that may impact energy
intake (274) and may use food and diet to address their negative perceptions of body image (281). Thus, our objective was to investigate the effect of TVV while eating and pubertal status on subjective appetite and mealtime FI 30 min after consumption of a glucose (1.0 g/kg body weight) or non-caloric sweetened drink in 9 – 14 y old girls. Our hypothesis was that TVV reduces the effect of subjective feelings of satiety arising from a glucose drink and increases mealtime FI in peri-pubertal and post-pubertal girls.

6.3 SUBJECTS AND METHODS

6.3.1 Subjects

Girls aged 9 – 14 y participated. The Human Subjects Review Committee, University of Toronto Canada, Toronto District School Board and Toronto Catholic District School Board approved this study. Study population and recruitment strategies were similar to those reported previously (206, 228, 282, 283). A screening was scheduled for the eligible child and parent at the Department of Nutritional Sciences where informed written consent was obtained from the parent and written assent from the child. Height (m) and weight (kg) were measured, and age- and-sex specific BMI percentiles were calculated according to the Canadian terminology (8) of the CDC and Prevention 2000 growth charts (284). Body fat content (%) was estimated by measures of triceps, biceps, supra-ilial and subscapular skinfold thickness (mm) using Harpenden skinfold calipers (Cambridge Scientific Industries, Cambridge, Maryland, USA). The sum of 4 skinfold measurements was used to estimate percent fat mass from age- and sex-specific regression equations (280), as previously described (206, 228, 282, 283). Girls who began menstruating were categorized as post-pubertal, while all other girls were categorized as
peri-pubertal. Girls were familiarized with visual analogue scale (VAS) questionnaires used in the study and asked to select the type of pizza they would like to eat during test visits.

6.3.2 Study Design

A within-subject, repeated measures design was used to examine FI after either the glucose or control drink, with or without TVV. Girls were randomly assigned to a treatment order, which was counterbalanced. On four separate mornings, 7 d apart, girls were given equally sweetened drinks of a SPLENDA® Sucralose control or 1.0 g/kg body weight glucose in 250 mL of water, 2 h after a standardized breakfast of Parmalat® fat-free skim milk (250 ml, 91 kcal), Honey Nut Cheerios® (26 g, 103 kcal donated by General Mills, Inc.) and Tropicana Orange Juice® (236 ml, 110 kcal). Thirty minutes after drink consumption, girls were provided with an ad libitum pizza lunch, with or without TVV for 45 min, and instructed to eat until comfortably full. In the TVV condition, the program Hannah Montana started simultaneously with the placement of a pizza tray in front of the subjects.

6.3.3 Protocol

Procedures are similar to those reported previously (206, 228, 282, 283). Girls arrived at the Department between 0900 and 1200, 2 h after consuming the standardized breakfast at home. VAS were used to measure subjective feelings assessing motivation-to-eat and physical comfort.
and were administered at baseline (0 min), at regular intervals pre-meal and immediately post-meal. Sweetness and palatability of test beverages/pizza lunch VAS were administered immediately after drink consumption or post-meal. Motivation-to-eat VAS, which measure dimensions of subjective appetite (269), was composed of four 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 full do you feel? (“not full at all” to “very full”), and (4) How much food do you think you can eat? (prospective food consumption (PFC)) (“nothing at all” to “a large amount”) (206, 228, 282, 283). Girls were instructed to read each question and place an “x” along the 100 mm line depending on their current feelings. Physical comfort was assessed by “How well do you feel?” (“not well at all” to “very well”). Sweetness and palatability VAS were formatted similarly (206, 228, 282, 283).

Two equally sweetened drinks were used. One contained 1.0 g/kg body weight glucose monohydrate (Grain Process Enterprises, Toronto, ON Canada) and the other was a control drink, matched for sweetness and flavor by addition of the high-intensity sweetener SPLENDA® Sucralose (donated by Tate and Lyle Sucralose, Inc. Deautur, IL), since it is not metabolized in the body and does not alter blood glucose or insulin secretion (270). Aspartame-sweetened, orange-flavored crystals (1.1 g, Sugar Free Kool-Aid, Kraft Canada Inc., Don Mills, ON Canada) were added to each drink to standardize flavor. Drinks were prepared 1 day prior to consumption in covered, opaque cups and stored in the refrigerator. The following morning, subjects consumed the chilled drinks, followed by 50 mL of water to minimize aftertaste. After drink consumption, girls engaged in child appropriate entertainment (cards, board games, etc.). Thirty minutes after drink consumption, subjects were escorted to a feeding room with individual
cubicles and served an *ad libitum* pizza lunch along with a 500 mL bottle of water (Danone Crystal Springs) for 45 min.

Girls were asked to remain seated for the 45 min duration and were instructed to eat until “comfortably full.” A freshly baked tray of pizza was provided every 15 min starting 30 min after drink consumption. Two varieties of Deep ‘N Delicious 5” diameter pizza (~ 200 kcal each) were used; pepperoni and three-cheese pizzas were donated by McCain (McCain Foods Ltd, Florenceville, NB, Canada). Each tray contained 3 pizzas: 2 of their first choice and 1 of their second choice. Cooked pizzas were weighed and cut into four equal pieces before serving, and the amount left after the meal was subtracted from the initial weight to measure FI. Each variety of pizza was weighed separately and energy consumed (in kcal) was calculated using manufacturer information. Bottled water was weighed before and after the meal to calculate the net amount ingested. Post-meal, girls rated their appetite, physical comfort and pleasantness of the meal.

During each of the TVV conditions, while eating, girls watched two of four episodes of Hannah Montana (Vol 01, Livin’ The Rock Star Life! Walt Disney Studios Home Entertainment) on a 15” liquid crystal display television (Sharp Aquos, model number: LC-15B6U, Mahwah, New Jersey, USA). Headphones were provided (Sony, model number: MDR-V250) to prevent distraction from other girls in adjacent cubicles. Each child watched, in random order, all four shows during the study. TV programs excluded food messages and advertisements and were on only during mealtime. Following the two TVV conditions, children filled out a TVV VAS to assess their enjoyment of the program with the following question: “How well did you enjoy the program?” (“not well at all” to “very well”).
Eating behaviour assessment

The Dutch Eating Behaviour Questionnaire was administered to assess restrained eating (scores range from 1.0 – 2.4) (268). Younger participants received assistance if they had difficulty interpreting the questionnaire’s language.

6.3.4 Statistical analyses

One-tailed tests were used to determine differences in baseline subject characteristics between groups because we hypothesized *a priori* that age, body weight, height, BMI percentile, fat mass, fat free mass and restraint would be significantly higher in post-pubertal vs. peri-pubertal girls. Food and water intake, physical comfort and palatability of the meal were analyzed using a within-subjects repeated measures 3 x 2 factorial design using the PROC MIXED procedure with drink (control vs. glucose), TVV (no TVV vs. TVV) and pubertal status (peri-pubertal vs. post-pubertal) as main factors. Within the peri-pubertal and post-pubertal girls, a repeated measures 2 x 2 factorial design using the PROC MIXED procedure with drink (control vs. glucose) and TVV (no TVV vs. TVV) was used. Caloric compensation, which is the amount of food compensated for after the caloric drink, was assessed using a paired two-tailed t-test. Subjective appetite (reported as the change from baseline) was split into pre-meal (0 - 30 min) and post-meal (30 - 75 min) periods. The pre-meal period was assessed using drink, pubertal status and time as factors. TVV was not included in the pre-meal model as TVV occurred at mealtime only. The post-meal period was assessed using drink, TVV and pubertal status as factors. Drink palatability and sweetness, and TVV enjoyment were assessed using
drink and pubertal status as factors. Post-hoc analysis by the Tukey-Kramer test was performed when treatment effects were found to be statistically significant.

An average appetite score was calculated at each time of measurement for each drink treatment by the formula:

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

which reflects the 4 questions on the motivation-to-eat VAS as used previously (225, 245, 285, 286). FI was determined from the total energy content of the pizza consumed at the meal. Cumulative energy intake was calculated from the sum of calories from the drink and meal. Percent caloric compensation was calculated for each person by the following formula (206, 224, 228):

\[ \text{Compensation} \% = \frac{\text{Control intake (kcal)} – \text{Treatment intake (kcal)}}{\text{kcal in treatment drink}} \times 100. \]

Data are presented as means ± standard error of mean (SEM). Significance was considered at \( p < 0.05 \). Correlations on dependent measures were conducted by use of Pearson correlation coefficients. Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc., Carey, NC) was used for statistical analyses.

6.4 RESULTS

6.4.1 Subjects

Twenty-five girls participated (Table 6.1). Twenty-one girls were between the 15th and 85th (NW), three were between the 85th and 95th (overweight), and one was greater than the 95th (obese) age- and sex- specific BMI percentiles (8, 284).
Table 6.1: Baseline characteristics of test subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All girls</th>
<th>Peri-pubertal</th>
<th>Post-pubertal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11.5 ± 0.4</td>
<td>10.4 ± 0.2*</td>
<td>13.8 ± 0.1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>42.1 ± 2.2</td>
<td>36.3 ± 1.7*</td>
<td>54.3 ± 2.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>148.8 ± 2.1</td>
<td>143.4 ± 1.8*</td>
<td>160.3 ± 1.7</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>55.8 ± 5.2</td>
<td>50.8 ± 6.4</td>
<td>66.3 ± 8.3</td>
</tr>
<tr>
<td>Fat mass§ (%)</td>
<td>26.7 ± 0.9</td>
<td>25.5 ± 1.0*</td>
<td>29.2 ± 1.5</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>73.3 ± 0.9</td>
<td>74.5 ± 1.0*</td>
<td>70.8 ± 1.5</td>
</tr>
<tr>
<td>Restrained eating†</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.1*</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

*Significantly different from post-pubertal by unpaired t-test (p < 0.05).

Data are presented as means ± SEM. (n = 25; post-pubertal, n = 8 and peri-pubertal, n = 17).
§Fat mass determined from the sum of skinfold measurements at four points (18).

†Restrained eating was measured with the Dutch Eating Behaviour Questionnaire (21).

6.4.2 Food and water intake

Drink (p < 0.001), but not TVV or pubertal status affected FI at the meal (Table 6.2). There was a drink-by-pubertal status interaction (p = 0.028), explained by a 27% reduction in FI by glucose in post-pubertal girls, but only 12% in peri-pubertal girls, independent of TVV (Figure 6.1A). There were no correlations between BMI or TV program enjoyment and FI.

Cumulative energy intake was not affected by drink, TVV or pubertal status (Table 2). Water intake was not affected by glucose or TVV, but was greater in post-pubertal vs. peri-pubertal, girls (Table 6.2; p < 0.001).

Table 6.2: Effect of glucose drink, TVV and pubertal status on food intake, cumulative energy intake and water intake

<table>
<thead>
<tr>
<th>Drink</th>
<th>TVV</th>
<th>Pubertal status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Glucose</td>
</tr>
<tr>
<td>Food intake§ (kcal)</td>
<td>940 ± 39</td>
<td>779 ± 31*</td>
</tr>
<tr>
<td>Cumulative energy intake† (kcal)</td>
<td>940 ± 39</td>
<td>948 ± 32</td>
</tr>
</tbody>
</table>
†Data are means ± SEM; (n = 25). A 3-factor ANOVA for food intake, cumulative energy intake and water intake was used with drink, TVV and pubertal status as main factors. *Significantly different (p < 0.001).

§Food intake was affected by drink (p < 0.001), but not TVV (p = 0.194) or pubertal status (p = 0.595) and a drink-by-pubertal status interaction (p = 0.028).

†Energy from drink plus energy from test meal intake. Cumulative energy intake was not affected by drink (p = 0.778), TVV (p = 0.268) or pubertal status (p = 0.360) or by interactions among the factors (p > 0.05).

‡Water intake at the pizza meal was not affected by drink (p = 0.932), TVV (p = 0.278), but affected by pubertal status (p < 0.001).

In peri-pubertal girls (n = 17), there was an interaction between the glucose drink and TVV (p < 0.017), demonstrated by a 22% reduction in FI in the no TVV condition and only a 1% reduction in the TVV condition (Table 6.3, Figure 6.1B). Cumulative energy intake in peri-pubertal girls was not affected by drink or TVV alone, but there was a drink-by-TVV interaction (Table 3; p = 0.017). However, post-hoc analysis was unable to detect significant differences between treatments.

Table 6.3: Effect of glucose drink and TVV on food intake and cumulative energy intake in peri-pubertal and post-pubertal girls

<table>
<thead>
<tr>
<th>Drink</th>
<th>TVV</th>
</tr>
</thead>
<tbody>
<tr>
<td>208 ± 23</td>
<td>208 ± 22</td>
</tr>
<tr>
<td>223 ± 22</td>
<td>194 ± 23</td>
</tr>
<tr>
<td>146 ± 16</td>
<td>341 ± 23*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drink</th>
<th>TVV</th>
</tr>
</thead>
<tbody>
<tr>
<td>208 ± 23</td>
<td>208 ± 22</td>
</tr>
<tr>
<td>223 ± 22</td>
<td>194 ± 23</td>
</tr>
<tr>
<td>146 ± 16</td>
<td>341 ± 23*</td>
</tr>
</tbody>
</table>
### Peri-pubertal girls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glucose</th>
<th>No TVV</th>
<th>TVV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food intake§ (kcal)</strong></td>
<td>897 ± 50</td>
<td>792 ± 42*</td>
<td>819 ± 43</td>
<td>870 ± 50</td>
</tr>
<tr>
<td><strong>Cumulative energy intake† (kcal)</strong></td>
<td>897 ± 50</td>
<td>937 ± 43</td>
<td>892 ± 40</td>
<td>942 ± 52</td>
</tr>
</tbody>
</table>

### Post-pubertal girls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glucose</th>
<th>No TVV</th>
<th>TVV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food intake‡ (kcal)</strong></td>
<td>1030 ± 60</td>
<td>753 ± 42*</td>
<td>868 ± 66</td>
<td>915 ± 58</td>
</tr>
<tr>
<td><strong>Cumulative energy intake‖ (kcal)</strong></td>
<td>1030 ± 60</td>
<td>970 ± 46</td>
<td>976 ± 58</td>
<td>1024 ± 49</td>
</tr>
</tbody>
</table>

†Data are means ± SEM; (n = 17; peri-pubertal, n = 8; post-pubertal). A 2-factor ANOVA for food intake and cumulative energy intake was used with drink and TVV as main factors. *Significantly different (p < 0.05).

§Food intake in the peri-pubertal girls was affected by drink (p = 0.02), but not TVV (p = 0.388) and a drink-by-TVV interaction (p = 0.017).

†Energy from drink plus energy from test meal intake. Cumulative energy intake in peri-pubertal girls was not affected by drink (p = 0.374), TVV (p = 0.388), but there was a drink-by-TVV interaction (p = 0.017).

‡Food intake in the post-pubertal girls was affected by drink (p = 0.002), but not by TVV (p = 0.167) or a drink-by-TVV interaction (p = 0.968).

‖Energy from drink + energy from test meal. Cumulative energy intake in post-pubertal girls was not affected by drink (p = 0.295), TVV (p = 0.167), or a drink-by-TVV interaction (p = 0.968).

In post-pubertal girls (n = 8), glucose reduced FI by ~ 27% (p < 0.002) in both the no TVV and TVV conditions with no interaction between drink and TVV (Table 6.3). Cumulative energy intake in post-pubertal girls was not affected by drink or TVV, and there was no interaction between them (Table 6.3). In the no TVV condition, caloric compensation was 139 and 125% in the peri-pubertal and post-pubertal girls, respectively, while during TVV, caloric compensation was 16 and 127% in the peri-pubertal and post-pubertal girls, respectively (Table 6.4).
Figure 6.1: Interaction between drink composition and A) pubertal status and B) TVV in peri-pubertal girls. §Significantly different from post-pubertal (glucose); *Significantly different from all other treatments (Tukey-Kramer post hoc test, p < 0.005). Values are mean ± SEM (n = 17, peri-pubertal; n = 8, post-pubertal)
Table 6.4: Caloric compensation (%) in peri-pubertal and post-pubertal girls¶

<table>
<thead>
<tr>
<th></th>
<th>No TVV</th>
<th>TVV</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peri-pubertal girls</td>
<td>139 ± 31</td>
<td>16 ± 40</td>
<td>0.025</td>
</tr>
<tr>
<td>Post-pubertal girls</td>
<td>125 ± 36</td>
<td>127 ± 22</td>
<td>0.973</td>
</tr>
</tbody>
</table>

¶Data are means ± SEM; (n = 17; peri-pubertal, n = 8; post-pubertal). Paired t-test used between caloric compensation in the no TVV vs. TVV condition. Caloric compensation =\([(kcal \text{ consumed at the test meal after control drink} - \text{kcal consumed at the test meal after glucose drink})/(kcal \text{ in glucose drink})]\) × 100%.

6.4.3 Subjective appetite scores

Average appetite (Figure 6.2), desire-to-eat, hunger and PFC scores increased over time (p < 0.001) in the pre-meal (0 – 30 min) period, but were not affected by drink or pubertal status. Post-meal (75 min) minus pre-meal (30 min) average appetite means were reduced by the meal, but were not affected by drink (-44 ± 4 vs. -46 ± 4; control vs. glucose), TVV (-46 ± 4 vs. -44 ± 4; no TVV vs. TVV) or pubertal status (-47 ± 4 vs. -41 ± 3; peri-pubertal vs. post-pubertal). Similarly, desire-to-eat, hunger and PFC scores were reduced by the meal, but were not affected by drink, TVV or pubertal status.

Fullness was affected by time (p < 0.001, data not shown), and there was a trend for a decrease in fullness by glucose (p = 0.062) compared with control drink. A time-by-pubertal
status interaction showed that post-pubertal girls having a lower feeling of fullness at 30 vs. 15 min (p < 0.001). No factors affected fullness post-meal.

Figure 6.2: Effect of drink, pubertal status and time on pre-meal average appetite.
Conditions were Control, Post-pubertal (■), Glucose, Post-pubertal (▲), Control, Peri-pubertal (□) and Glucose, Peri-pubertal (△). Time affected average appetite (3-way ANOVA, Tukey-Kramer post hoc test, p < 0.001). (n = 17, peri-pubertal; n = 8, post-pubertal).

6.4.4 Subjective ratings of physical comfort, sweetness, pleasantness and enjoyment of the TV program

There were no main effects of drink, TVV or pubertal status on physical comfort and pleasantness of the meal, and no effect of drink and pubertal status on pleasantness and
sweetness of the drink (data not shown). However, TV program enjoyment was affected by pubertal status, with peri-pubertal girls rating the programs 31% higher than post-pubertal girls (p = 0.005), but not by drink or drink-by-pubertal status.

6.4.5 Associations between subjective appetite and food intake

In peri-pubertal girls, average appetite, desire-to-eat, hunger, fullness and PFC were not associated with FI at 30 min. In post-pubertal girls, average appetite (r = 0.74, p = 0.037), desire-to-eat (r = 0.85, p = 0.007) and hunger (r = 0.76, p = 0.027) were positively associated with FI at 30 min, while PFC (r = 0.46, p = 0.247) and fullness (r = -0.38, p = 0.348) were not.

6.5 DISCUSSION

Overall, TVV had no effect on FI at the pizza meal. However, in peri-pubertal girls, TVV in the mealtime environment prevented the reduction in FI after the glucose drink.

Lower lunchtime FI 30 min after glucose in the no TVV condition is consistent with studies in NW adult men (285) and children (206, 228, 282, 283). However, there may be several explanations for why peri-pubertal, but not post-pubertal, girls failed to decrease FI after the glucose drink while TVV. First, both age and puberty may be factors. Post-pubertal girls had an average age of 13.8 y compared with 10.4 y in peri-pubertal girls, and responded similar to 15 – 16 y olds, in which no significant differences were found in FI between TVV, no TVV and listening to music (204). Reduction in FI after glucose was more robust in post-pubertal girls, indicating they may have a stronger physiological response to satiety signals than peri-pubertal girls. Furthermore, with the onset of puberty, estrogen exerts physiological functions including affecting FI regulation and the action of intake regulatory hormones including ghrelin and
cholecystokinin (CCK) (126). In rats, estradiol increases CCK’s satiating effects (124, 125) and attenuates ghrelin’s orexigenic effects (119), leading to lower FI. Energy intake is decreased in the follicular compared to the luteal phase of the menstrual cycle due to higher levels of estrogen (121), which may have contributed to the greater reduction in FI in post-pubertal girls after glucose. Although we did not account for menstrual cycle phase, we were not powered to evaluate the effects of FI and phase with the current study design.

In contrast to the decrease in FI after glucose in both groups in the no TVV condition, appetite did not decrease and fullness did not increase in either peri-pubertal or post-pubertal girls, which is consistent with other reports in children (206, 225, 228, 283). While this may suggest that children are unable to accurately reflect their feelings of appetite using VAS, they consistently report reductions in desire-to-eat, hunger and PFC and increased fullness after the test meal (206, 225, 228). Because average appetite, desire-to-eat and hunger were positively associated with FI at 30 min in post-pubertal girls only, it can be suggested that subjective appetite is a learned association with FI in post-pubertal girls, but peri-pubertal girls are yet unable to make this association (181). However, fullness was not correlated to FI at 30 min, suggesting this measure may be a weak predictor of FI in girls.

Surprisingly, TVV did not delay satiation in either peri- or post-pubertal girls, which is in contrast to the higher energy intakes observed in 9 - 14 y old boys (206), undergraduate students (201) and women who watch TV during mealtime (200). This latter effect of TVV is attributed to diminished awareness of the amount of food consumed (287), as a result of disrupted cognitive restraint (288) and habituation to food cues (205).

Cognitive restraint may have been a factor in post-pubertal girls, since scores were significantly greater compared to peri-pubertal girls. Restraint affects satiation through
restriction of FI as a means of controlling body weight (289). Self-regulation of FI is evident following menarche in 12 – 17 y old girls, who limit their FI due to concerns with body image (281). Although restraint associates with reduced FI in adult females (290) and restraint was higher in post-pubertal girls, its role is unclear because no correlation was found between restraint and FI. Similarly, restraint does not explain why TVV did not delay satiation in peri-pubertal girls. An alternative explanation may be greater sensitivity to distraction.

TVV as a distraction is known to disrupt habituation to food cues, particularly when there is active engagement in the stimulus (attention allocation), leading to greater energy intakes (205). Consistent with this, TV program enjoyment was higher in peri-pubertal girls, and children (9 – 12 y old) ate more of a preferred snack and spent more time eating when watching a continuous TV program that required attention allocation compared to a repeated segment of a program and no TVV (205). Although we did not observe greater FI with TVV in peri-pubertal girls, distraction may have been a factor in their failure to reduce their FI in response to glucose and at the pizza meal.

Because girls were selected from a relatively large age range and varied considerably in body weight, glucose was provided on a body weight basis. Peri-pubertal girls and post-pubertal girls had an average intake of glucose of 36 and 54 g, respectively. Full compensation for the energy content of the glucose without TVV was found in both peri-pubertal and post-pubertal girls (139 and 125%, respectively) consistent with previous reports showing children compensate in later meals for sugars consumed as liquids, suggesting that sugars do not bypass physiologic regulatory systems (219, 291), unless there are environmental distractions present (206). While TVV, peri-pubertal girls compensated by ~ 9 kcal, while post-pubertal girls compensated by ~ 276 kcal. However, the differing amounts of glucose are unlikely to be a factor affecting the
differences in the effect of TVV on glucose-induced FI suppression because neither the number of calories in the glucose drink nor body weight were correlated with caloric compensation after glucose in the TVV condition.

Our study has limitations that are worth noting. First, in place of Tanner staging, we classified girls as peri-pubertal and post-pubertal by the onset of menarche, and as such, may have failed to uncover whether each specific stage (I, II, III, IV) had any impact on response to the glucose or TVV. Nevertheless, our classification system identified a unique response between the peri-pubertal and post-pubertal girls. Second, the sample size was unbalanced between the two groups. Although only 8 post-pubertal girls were included in the analysis, there was adequate power (> 80%) to detect a difference of ~ 277 kcal between treatments with a standard deviation of 208 kcal, supporting the validity of the results observed in the post-pubertal group. Third, it is not possible to generalize our results to all TV programs. Lastly, these results show that pubertal status is a significant factor to consider when studying the effect of environmental factors on FI control in adolescent girls.

6.6 CONCLUSION

In conclusion, TVV at mealtime reduced caloric compensation following consumption of a glucose drink in peri-pubertal, but not post-pubertal, girls, but had no effect on satiation as measured by FI at the meal. TV is a modifiable part of the mealtime environment, and further studies are warranted to investigate whether avoidance of mealtime TVV will improve sensitivity to physiologic cues arising from food ingestion during peri-pubertal development.
7 GENERAL DISCUSSION

The results of this research support the overall hypothesis that physiological and environmental variables are both independent and interactive in determining FI in children during puberty. These studies further an understanding of how physiological signals of satiety (absence of hunger) and satiation (factors within a meal that bring eating to an end) regulate short-term FI and energy balance in children and adolescents. Furthermore, these studies provide novel information on how pubertal development and obesity alter appetite hormone responses in adolescents, how pubertal stage affects FI response in boys and girls and how distraction in the mealtime environment interact with physiological factors to diminish satiety signals from a pre-meal glucose drink.

The interrelationships among pubertal status, body weight, and sex with the mealtime environment provides several lines of evidence that physiological variables interacted with environmental factors to determine FI in response to glucose and WP beverages. First, mid-late pubertal obese males had greater change in PYY and lower ghrelin compared to pre-early pubertal obese males which may have implications for FI during this developmental period (Chapter 4). Second, mid-late compared to pre-early pubertal boys had greater FI, but not when FI was corrected for body weight, while FI/kg body weight was lower in mid-late than pre-early pubertal girls. Third, the response to carbohydrate, but not protein drinks decreased from 30 to 60 min in mid-late pubertal male and female children (Chapter 5). Fourth, TVV disrupted satiety
signals from a glucose drink in peripubertal, but not postpubertal, girls (Chapter 6). Lastly, although obese adolescents had impaired appetite hormone responses compared to NW controls (Chapter 4), FI was not affected by body weight in response to caloric beverages given relative to body weight (Chapter 5).

A novel finding of our research was that pubertal stage affected the action of intake regulatory hormones PYY and ghrelin, FI in males and females and caloric compensation to pre-meal glucose drinks over time. These results emphasize that puberty is a critical time period involved in the regulation of FI in children. Alterations in FI regulation during the development of puberty may be due to several factors. First, the regulation of short-term FI has been reported to be less precise with increasing age (217, 220, 221, 250, 292). Second, pubertal insulin resistance is a normal physiological event; mid-late pubertal children are more insulin resistant than pre-early pubertal children (147). Although changes in insulin concentrations between pre-early and mid-late pubertal obese males were masked by their fat mass (Chapter 4), insulin mediates the action of other FI regulatory hormones (17). Third, FI during pubertal development is largely a reflection of changes in body composition, with greater accumulation of fat free mass in males and greater increases in fat mass in females (273). Lastly, elevations in sex hormones during puberty can also affect the action of these hormones (126, 142); testosterone administration to peripubertal boys results in lower ghrelin levels (126). Thus, alterations in PYY and ghrelin in later puberty, as well as possible interactions with insulin resistance, leptin and sex hormones, may explain the different effects on FI and the lower compensation to glucose at 60 compared to at 30 min in the mid-late pubertal males and females. Unfortunately, while the present study suggests sex hormones during puberty may play a role, the cross-sectional and correlational design of the studies limits any causal conclusions that can be drawn about the
effects of pubertal development on FI. We are unable to dismiss the potential effect of other variables, such as sex differences in patterns of growth during puberty or the fitness of the child.

External stimuli in the mealtime environment reduced caloric compensation after a glucose drink given 30 min prior to a meal in boys (206) and in peripubertal, but not postpubertal, girls. TVV as a distraction in the mealtime environment disrupts habituation to food cues (205) and diminishes awareness of the amount of food consumed (287), leading to greater energy intakes. It is likely that peripubertal girls may be more sensitive to distraction (293) and failed to reduce their FI in response to glucose and at the test meal. In Chapters 5 and 6, compensation for glucose (0.75-1.0 g/kg body weight) at 30 min was similar between mid-late pubertal (122%) and postpubertal girls (125%, no TVV); however, compensation was less precise in the pre-early pubertal (73%) and peripubertal girls (139%, no TVV). The variable compensation scores for glucose may be explained by differences in pubertal stages (pre-early pubertal vs. peripubertal) (Chapter 6) or the inclusion of more overweight girls in study 2 (Chapter 5).

Another novel finding was that alterations in FI regulatory hormones in obesity do not translate into changes in short-term FI in obese adolescents. This observation is new in adolescents, but not in adults; hyperinsulinemic men decrease their FI after glucose preloads more than normoinsulinemic men and this response is associated with an enhanced response in satiety hormones (294). Consistent with a recent study that showed similar reductions in FI in NW and obese boys (295), our results suggest that there is no difference between overweight/obese and NW children in their response to satiety signals when provided with calories relative to their body size. This disconnect between appetite hormones and FI suggests that other satiety hormone mechanisms are involved in the regulation of FI; changes in peripheral
hormone responses may not directly affect short-term intake in children/adolescents and neural pathways affecting FI are extensive and difficult to interpret.

Protein was compared with carbohydrate because it is the most satiating of the macronutrients for adults (296, 297). However, a previous study in 9 – 14 y old boys found that a fixed dose (50 g) of protein suppresses FI less than carbohydrate in obese boys and to equal amounts in NW boys (228). This suggested that satiety signals from protein, but not carbohydrate, were diminished with body fatness (228). Furthermore, lower compensation to a fixed size carbohydrate pre-meal drink with increased body fatness has been shown in 3 – 5 y old girls (226). However, NW and overweight/obese boys and girls adjusted their intakes relative to their body weight when glucose and WP were provided on a body weight basis. In Chapter 5, the observed differences in FI between WP and glucose correspond to their glycemic and satiety hormone effects. Compared to glucose, pre-meal consumption of WP (10 and 20 g) led to lower pre- and post-meal blood glucose concentrations, but also of both insulin and C-peptide, indicating lower insulin secretion. Pre-meal consumption of WP led to higher plasma GLP-1 and PYY compared with pre-meal glucose. Similar to NW men (236), our findings indicate that WP does not increase cumulative energy intake in either boys or girls and may be a helpful strategy to maximize satiety and prevent excess intake during later meals in children.

Subjective appetite measures after consumption of the caloric beverages were affected by puberty and obesity. In study 1, average subjective appetite decreased after consumption of a caloric beverage in both pre-early and mid-late pubertal adolescents and in NW and obese adolescents; by 60 min post-drink, appetite was higher in mid-late compared to pre-early pubertal adolescents and in obese compared to NW adolescents. Lower appetite after the drink and pubertal differences were not observed in studies 2 or 3. This discrepancy may be due to the
composition of the beverage. In study 1, the combination of protein and carbohydrate may have provided a more sustained satiety hormone response compared to each alone (236). However, measures of appetite were positively associated with FI at 30 min in mid-late pubertal boys and girls in study 2, indicating that subjective appetite is a learned association with FI but that children in pre-early puberty are unable to make this association (181), as also found in study 3.

Although lower FI after caloric beverages consumed by children is consistent with previous reports that liquids do not bypass physiologic regulatory systems (219, 291, 298), subjective appetite is more accurately interpreted after solids than liquids in younger children (8-11 y, Appendix 8) (278, 299). The failure of appetite to decrease after the caloric drinks in studies 2 and 3 is consistent with other reports in children (228, 257). In contrast, lower average appetite (increased satiety) was found after caloric ad libitum pre-meal snacks of raisins, grapes and a mixed snack of raisins and almonds compared to water (278) and similarly after ad libitum after-school snacks of raisins, grapes, potato chips and chocolate chip cookies in NW children (299). This lack of sensitivity of subjective appetite to liquids in younger children merits further investigation.

In summary, the results of these studies provide a greater understanding of how physiological variables, both independently and through their interaction with environmental factors, determine how FI is regulated during pubertal development in children and adolescents.

7.1 Study Design: Strengths and Limitations

7.1.1 Strengths

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A major strength of the design was examining the effect of pubertal stage on appetite, intake regulatory hormones and FI in boys and girls. Many studies examining FI in children and adolescents fail to consider this stage in adolescence and its effects on energy intake regulation. Including pubertal stage as a factor in our study design allowed us to highlight a critical period of development that has implications for eating behaviours in children. Furthermore, the majority of studies that examine FI use only one sex, whereas in all but one study (Chapter 6), both boys and girls of varying body weights were included to assess the effect of sex on FI.

The preload paradigm used in our studies has several benefits: First, the design provided a framework to measure the role of physiologic signals affecting both satiety and satiation from previously consumed calories. Furthermore, the caloric preloads administered isolated the effect of glucose and WP alone, as well as in combination; this is necessary to examine specific macronutrient effects on appetite and FI.

7.1.2 Limitations

As noted earlier, the present study as designed fails to identify a direct role of sex hormones on energy intake regulation. Longitudinal studies throughout puberty are required to determine if there is an interaction between sex hormones, appetite hormones and FI. Furthermore, the children in Chapter 5 were self-staged using gender-appropriate Tanner drawings, (265, 266) and not examined by a physician trained in Tanner Staging. However, this was considered too invasive for healthy children recruited from the community. One of the limitations of study 1 (Chapter 4) was that FI after consumption of the mixed glucose and WP drink was not measured nor appetite hormones after glucose and WP drinks separately. This would have provided a more direct comparison of the relationship between appetite hormones
and subsequent FI. In addition, the acute and short-term effects of glucose and WP on FI, glycemia, and hormonal responses was assessed, therefore our results cannot be generalized to longer-term studies or overall daily caloric intake.

7.2 Significance and Implications

These novel experiments advance our understanding of physiologic and environmental factors, and the interactions among them, that contribute to energy imbalance during a crucial stage of development of appetite control in children. They provide a foundation for advice aimed at preventing and managing overeating in children and adolescents during puberty, as well as a basic understanding of FI regulation during pre-early and mid-late pubertal stages.

This research has implications for the development of recommendations based on scientific evidence for the development of health and nutrition programs aimed at targeting weight maintenance in NW children and weight loss in obese children during different stages of puberty. The results support recommendations for public policies and regulations to prevent and treat childhood obesity would include the development of leisure time schedules, which focus on reducing time spent eating while TVV in young girls. A greater understanding of how appetite hormones are regulated in NW and obese adolescents, as well as during puberty, provides initiative for the creation of eating plans that can be formulated to control appetite.

8 GENERAL SUMMARY AND CONCLUSIONS

Pubertal development and obesity alter appetite hormone responses in adolescents, pubertal stage affects FI response in boys and girls, and distraction in the mealtime environment
diminishes satiety signals from a pre-meal glucose drink in peri-pubertal, but not post-pubertal, girls.

In conclusion, physiological variables both independently and through their interaction with environmental factors, determine FI in children during puberty.

9 FUTURE DIRECTIONS

This research provided support that short-term FI and appetite regulation by children and adolescents was affected by physiological variables and their interaction with environmental factors during pubertal development. Thus, the results emphasize that puberty is a time of increased susceptibility to the development of obesity-related health issues as a result of the dramatic changes in physiology, body composition and energy demands associated with this process (10). However, further investigation is required to determine how puberty affects the long-term regulation of FI; significant alterations in energy intake occurring over a period of months or years will have implications for future eating patterns and body weight in adulthood. It is important to determine which stage of puberty has the greatest affect on FI in order to develop strategies to prevent excess energy intake. Furthermore, future research is needed to understand the specific effect of testosterone and estrogen on energy intake, insulin sensitivity and FI regulatory hormones by examining potential relationships between puberty, obesity and sex steroid concentrations. A longitudinal study measuring FI and appetite hormones over the development of puberty in children, including the measurement of sex hormone concentrations, body fatness and growth is needed to control for other factors that may affect energy intake during puberty.
Additionally, physiological mechanisms explaining the direct effect of pure fat sources on FI control require further investigation; consumption of high-fat foods can weaken satiety signals and motivate energy intake independent of energy need (300) and children prefer energy-dense foods that are richer in fat (243). The mu-opioid system is a key target for the hedonic experience of feeding and mu opioid receptor stimulation of the nucleus accumbens increases the intake of and preference for high fat foods (244). Thus, more research into food reward pathways using fMRI techniques will help identify how specific macronutrients affect FI in children. Combining neuroimaging, metabolic, and behavioral approaches are necessary to help improve our understanding of the physiological factors that influence FI in children.
10 REFERENCES


15. Rolls BJ, Roe LS & Meengs JS. Larger portion sizes lead to a sustained increase in energy intake over 2 days. *J Am Diet Assoc.* 2006 106 543-549.


30. Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC & Weigle DS. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J Clin Endocrinol Metab* 2004 **89** 1319-1324.


47. Misra M, Tsai PM, Mendes N, Miller KK & Klibanski A. Increased carbohydrate induced ghrelin secretion in obese vs. normal-weight adolescent girls. *Obesity* 2009 **17** 1689-1695.


79. Mittelman SD, Klier K, Braun S, Azen C, Geffner ME & Buchanan TA. Obese adolescents show impaired meal responses of the appetite-regulating hormones ghrelin and PYY. *Obesity* 2010 **18** 918-925.


86. Talsania T, Anini Y, Siu S, Drucker DJ & Brubaker PL. Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. *Endocrinology* 2005 **146** 3748-3756.


120. Eckel LA. The ovarian hormone estradiol plays a crucial role in the control of food intake in females. *Physiol Behav* 2011 104 517-524.


158. Pierroz DD, Catzelflis C, Aebi AC, Rivier JE & Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. *Endocrinology* 1996 **137** 3-12.


160. Pierroz DD, Gruaz NM, d'Alieves V & Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. *Neuroendocrinology* 1995 **61** 293-300.


181. de Graaf C & Kok FJ. Slow food, fast food and the control of food intake. *Nat Rev Endocrinol* 2010 **6** 290-293.


244. Zhang M, Gosnell BA & Kelley AE. Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. *J Pharmacol Exp Ther* 1998 285 908-914.


288. Bellisle F & Dalix AM. Cognitive restraint can be offset by distraction, leading to increased meal intake in women. *Am J Clin Nutr* 2001 *74* 197-200.


