Acute Inflammatory Response as a Mediator of the Effects of High-Intensity Exercise on Appetite and Food Intake in Boys.

by

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

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Abstract

The effect of high-intensity exercise (HIEX) and the mechanism by which it suppresses appetite and food intake (FI) is not well understood. The hypothesis of this research was that inflammation during and after HIEX plays a role. Three experiments were designed to investigate the hypothesis and to compare these mechanisms with the effects of macronutrient ingestion in male children. Experiment 1 investigated the effects of HIEX induced inflammatory- and stress biomarkers on appetite control in normal-weight (NW) and overweight/obese boys. HIEX resulted in reduced appetite that correlated with an increase in plasma concentration of IL-6 with no effect of body-weight. However, while a role for IL-6 in the response can be suggested, the suppression of appetite could have been mediated by the associated decrease in active ghrelin and/or increase in cortisol. Experiment 2 further examined the role of IL-6 in exercise-induced anorexia in NW children. HIEX reduced subjective appetite but not FI, increased IL-6 and cortisol and decreased active ghrelin and blood glucose. An independent role for IL-6 in appetite suppression was not supported. However, IL-6 response was associated with active ghrelin and
cortisol, thus potentially mediating appetite via these interactions. Experiment 3 was a study of the role of IL-6 and TNF-α in satiety and appetite after consumption of glucose or protein beverage by boys. Neither affected an immediate response in plasma IL-6 and TNF-α concentrations. However, in the pooled data, mean levels of IL-6 were strongly correlated with later FI. Thus, this research provides support for further examination of the role of IL-6 in appetite control.
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Throughout my PhD I have received a great deal of support and assistance. First, I would like to thank my supervisor and colleague, Dr. Harvey Anderson. You were invaluable during these years, your wisdom, experience and guidance are the reason I got to where I am today.

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<td>AgRP</td>
<td>Agouti-Related Protein</td>
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<tr>
<td>BG</td>
<td>Blood Glucose</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>BPM</td>
<td>Beats Per Minute</td>
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<tr>
<td>BW</td>
<td>Body-Weight</td>
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<td>CCK</td>
<td>Cholecystokinin</td>
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<td>CNS</td>
<td>Central Nervous System</td>
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<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>DTE</td>
<td>Determination To Eat</td>
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<td>EB</td>
<td>Energy Balance</td>
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<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
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<td>Exercise</td>
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<td>HIEX</td>
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<td>HIOMA-IR</td>
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<tr>
<td>IL-6</td>
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<td>RPM</td>
<td>Revolutions Per Minute</td>
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Chapter 1
INTRODUCTION

The mechanisms of food intake (FI) and body-weight (BW) regulation are not well understood. Several models have been proposed over the past 60 years to explain how energy balance (EB) and stable BW is achieved. These models are under consistent revision and bear little resemblance to those proposed in the 1950s. A major focal point in the early models of appetite control was the idea of energy homeostasis, with several feedback signals that stimulate compensatory adjustments via a central control system. However, the regulation of EB is often viewed as asymmetrical (J. E. Blundell & Hill, 1985), where the reduction in BW is strongly protected against, but BW is not tightly regulated (Lenard & Berthoud, 2008). Thus it is not surprising that a trend analysis of several National Health and Nutrition Examination Survey (NHANES) surveys included data from 63,761 adults aged 20-74y showed an average increase of intake of 240 kcal/day from 1971-1975 to 2009-2010 (Ford & Dietz, 2013), contributing to a positive EB and consequently obesity (OB).

Early theories of appetite control focused on the effect of macronutrients on appetite and FI control, starting with the glucostatic (Mayer, 1952), aminostatic (Mellinkoff, Frankland, Boyle, & Greipel, 1956) and lipostatic (Mayer, 1955) theories in the 1950s, followed by a focus on catecholaminergic and serotonergic regulation of appetite. One different theory of appetite/EI control that did not focus on macronutrients was investigated over 60 years ago. This concept of appetite control sought to establish a fundamental relationship between energy expenditure (EE) and EI. EI and EE are well known to be linked (Edholm, 1977; Edholm, Fletcher, Widdowson, & McCance, 1955; N. B. Marshall & Mayer, 1954; Mayer, 1955). Edholm et al. suggested that Els
reflect EEs (Edholm et al., 1955), to prevent starvation under conditions of increased EE. In 1956, Mayer et al. showed that FI is increased with PA, matching EE and regulating BW accurately, but in individuals with low PA levels, FI is higher than expenditure resulting in the accumulation of adipose tissue and eventually overweight (OW) and obesity (OB). In industrialized countries, EE has been decreasing over the past five decades by 100-200 kcal/day. This decrease associates with positive EB and OW and OB. In Canada, 61.8% of male (8.2 million) and 46.2% of female (6.1 million) adult Canadians found to be OB or OW and 30% of Canadian children aged 2-17 years have been found to be OW or OB, with 18.3% being OW and 10.6% being OB (StatisticsCanada, 2019). Another report from 2010 estimated that the OB/OW related Canadian Health Care costs reach about 6 billion dollars or 4.1% of Canada’s Health Care Expenditures, in addition, EE has also been decreasing dramatically (P. M. Anderson & Butcher, 2006).

EE is defined as the sum of internal heat produced and external work. The internal heat produced is, in turn, mainly a sum of basal metabolic rate (BMR) and the thermic effect of food, whereas the external work is comprised out of the resting EE and physical activity (PA). PA includes exercise (EX) and incidental activity integrated into daily activity (Organization, 2019). It is hypothesized the habitual PA and EX affect appetite control mechanisms and contribute to normal regulation of BW beyond the pure cost of EE (Tremblay et al., 1986). In adults, EX interventions with approximately 3 to 16 days of moderate EX lead to increased FI in normal-weight (NW) individuals (Melzer, Kayser, Saris, & Pichard, 2005), without reaching full compensation for EE (J. E. Blundell, Stubbs, Hughes, Whybrow, & King, 2003). The mechanisms of how compensation with increased EE occurs still need to be elucidated.
More recent theories in appetite control focus on a more central control of appetite. In the past two decades, the peptide hypothesis of central control of appetite has added to the ‘somewhat dated’ aminergic ideas. The neuropeptide model of appetite control hypothesizes that EB is largely regulated by the neurons in the central nervous system (CNS), involving many peripheral humoral, and neural signals. This network is sensitive to short and long-term changes in energy requirements. It acts to control the timing of meals, their size and duration, and possibly, under some circumstances, their composition. The metabolism of ingested food is closely linked to food consumption and is also subject to neural regulation (Wilding, 2002). Given the large number of potential signals involved in the regulation of EB, a complex integrating system has evolved, with the hypothalamus playing a key role. However, to this date a single reliable biomarker of appetite has not been identified (de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). A link between EX and appetite/FI could not be established, despite the constant efforts of neurologists, EX physiologists, nutritionist and countless other researchers.

However, high-intensity (HIEX) represents a special condition where EE is not partially compensated for by increased FI and even decreased. Appetite after HIEX is suppressed (N. A. King, Burley, & Blundell, 1994; Thompson, Wolfe, & Eikelboom, 1988) up to 60 mins’ post-EX (N. A. King et al., 1994) with some studies also showing a suppression of FI (N. A. King et al., 1994; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). EX intensity is defined as a percentage of maximum or peak oxygen consumption (VO$_2$max or VO$_2$peak), sedentary- (<20% VO$_2$max), low - (20-40% VO$_2$max), moderate- (40-60% VO$_2$max), HIEX- (60-85% VO$_2$max) and very HIEX (> 85% VO$_2$max) (Pescatello & American College of Sports Medicine., 2014). Studies investigating the effects behind this “EX induced anorexia” have focused on the neuropeptide control of appetite
model (Deighton, Barry, Connon, & Stensel, 2013; Larson-Meyer et al., 2012; Schubert, Sabapathy, Leveritt, & Desbrow, 2014; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Unick et al., 2010). However, these studies often display contradicting and inconsistent results perhaps because the peptide hypothesis of appetite control is entirely based on the sensing of nutrients in the gut and does not reflect the metabolic changes induced by EX. Gastric activation occurs at the sight, smell, or mere thought of food (cephalic phase), through the stimulation of stretch- and chemical receptors after food is being ingested (gastric phase), and upon the arrival of partially digested proteins and amino acids in the duodenum (intestinal phase) (Johnson, 2012). These appetite-regulating signals include anorexic hormones or appetite-suppressing signals from the small intestine, including, and from the stomach, leptin and the only known appetite-stimulating signal, ghrelin (G. H. Anderson, Hunschede, Akilen, & Kubant, 2016).

In contrast, PA stimulates many other physiologic systems that may affect EI. The inflammatory and stress responses have potent appetite suppressing effects and are also upregulated with EX, increasing at high intensities. Several animal models have identified IL-6 and TNF-α as potential appetite regulators (Banks, 2001; Kasapis & Thompson, 2005; Morley, Thomas, & Wilson, 2006; Wallenius, Wallenius, Sunter, Dickson, & Jansson, 2002). However, the role of these biomarkers of inflammation on appetite regulation in humans has received little investigation.

Therefore, the objective of this research is to begin elucidating an understanding of the role of inflammation in mechanisms by which HIEX affects appetite and FI in male children and adolescents.
Chapter 2
LITERATURE REVIEW

2.1. Introduction

To provide background for this research, the following literature review is composed of seven sections. After a brief introduction on Fl regulation, the relationship between habitual physical activity, EX interventions, acute EX and appetite is examined. This is followed by a review of the mechanisms of appetite regulation with EX, and a section exploring the effects of HIEX on the inflammatory and stress systems. The final section provides a summary of the physiological mechanisms of HIEX on appetite and Fl and possible mechanisms.

OW and OB have developed into a worldwide epidemic (Jaacks et al., 2019). Currently, 59% of adult Canadians are either OW or OB, and 29% of adolescents have unhealthy weights, translating into seven million Canadians living with OW/OB (Janssen, 2013). A report from 2017 estimated that the OB/OW related Canadian Health Care costs reach 6 billion dollars or 4.2% of Canada’s Health Care Expenditures. However, these expenditures only account for costs related to OB, and not for a loss in productivity, reductions in tax revenues or psychosocial costs (Canada, 2017). Especially in children, OB/OW has severe immediate and long-term implications for health and well-being. OW/OB children have a higher risk for cardiovascular diseases, such as higher levels of cholesterol and blood pressure. Approximately 70% have at least one risk factor for cardiovascular diseases (Umer et al., 2017), are more likely to be pre-diabetic and more prone to develop bone and joint problems as well as sleep apnea, and social and psychological problems such as stigmatization and poor self-esteem (Sahoo et al., 2015).
2.2. Food Intake Regulation

The current models of appetite control are based on the hypothesis that the brain can detect alterations in energy stores and generate metabolic and behavioural responses to maintain EB. Eating behaviour is motivated by hunger, cravings and hedonic sensations, designed to control energy homeostasis. The idea that the brain is involved in appetite and FI was first formulated in patients with pituitary tumours encroaching on the base of the brain. These patients displayed “adiposogenital syndrome”, patients with this syndrome displayed extreme appetite, morbid OB, and hypogonadism (Elmquist, Elias, & Saper, 1999). The same characteristics were shown in rats that had lesions in the ventromedial hypothalamus (Hervey, 1959). In contrast, rats with lesions in the lateral hypothalamus showed decreased FI and even starvation (Hervey, 1959). This formed the “dual center model” of FI regulation, where the ventromedial hypothalamus contains the satiety centre and the lateral hypothalamus the feeding centre of the brain. However, surgical lesions at the time were imprecise and damaged other regions and nerves as well (Elmquist et al., 1999). Leptin’s primary secretion site are adipocytes, and its concentration in plasma correlate with adipose tissue and levels of OB. (Ahima, Saper, Flier, & Elmquist, 2000; Myers, Cowley, & Munzberg, 2008).

With the discovery of appetite hormones, the hypothesis emerged of a gut-brain axis of control linking the gastrointestinal tract (GI tract) and the central nervous system (CNS). This connection has been investigated using surgical and chemical approaches (G. J. Schwartz, 2000). Early studies established a negative feedback control of vagal afferent innervation on feeding. For example, several experiments showed that balloon distension, vagal stimulation, and infusions of carbohydrate-, fat-, and protein solutions induced satiety and/or reduced meal size.
This effect can be blocked by applying capsaicin directly to the vagus nerve (G. J. Schwartz, 2000; G. P. Smith, Jerome, & Norgren, 1985; South & Ritter, 1988). On the other hand, surgically severing the vagus nerve from the gut increases meal size and feeding duration (G. P. Smith et al., 1985). Vagal signalling in the brainstem and hypothalamus also arises from GI hormones. The first satiety signal identified from the gut was CCK (Liebling, Eisner, Gibbs, & Smith, 1975). CCK decreases meal size (Kraly, Carty, Resnick, & Smith, 1978; Liebling et al., 1975) and CCK-1 receptor antagonists can increase feeding in fed rats (Bi et al., 2007). Glucagon-like-peptide-1 (GLP-1) is secreted from the L-cells when nutrients arrive in the intestine (Baggio & Drucker, 2007), and GLP-1 injections in the periphery or the brain decrease FI (Baggio & Drucker, 2007; K. G. Murphy & Bloom, 2006). Peptide-Y (PYY) is co-secreted with GLP-1 and also decreases FI by inhibiting agouti-related protein/neuropeptide Y (AgRP/NPY) neurons in the hypothalamus (Batterham et al., 2002; Ghamari-Langroudi, Colmers, & Cone, 2005). Ghrelin is a 28-amino acid peptide, predominately synthesized in the stomach (Tschop, Smiley, & Heiman, 2000; Wiedmer, Nogueiras, Broglio, D'Alessio, & Tschop, 2007). It increases in a fasted state and prior to meals in humans and is a critical hunger signal. Direct injections of ghrelin into the brain or periphery induce hunger and ultimately feeding (Tschop et al., 2000).

The process of appetite and FI regulation is complex and encompasses many central and peripheral control mechanisms (Figure 2.2.), reflecting an interplay between hunger, satiety, and satiation. Satiation is defined as the drive to terminate eating at any point after the onset of a meal. It is the one signal that interacts with both positive and negative feedback signals. Satiety describes a negative feedback process that is initiated after the termination of a meal. These appetite-regulating signals arise primarily through gastric activation. Gastric activation occurs at
the sight, smell, or mere thought of food (cephalic phase), through the stimulation of stretch- and chemical receptors after food is ingested (gastric phase), and upon the arrival of partially digested proteins and amino acids in the duodenum (intestinal phase). This negative satiety feedback signal is active until the positive feedback signal, which initiates hunger is overpowering satiety.

A research focus on control of appetite and FI developed in the early 1950s. It was first hypothesized that differences in FI must originate in the differences in EE (Edholm et al., 1955). However, this hypothesis was replaced by the glucostatic-, lipostatic-, and aminostatic theories of appetite control (Mayer, 1955; Mellinkoff et al., 1956), which recognized that food ingestion provided signals to the brain based on its composition. These nutrient focused hypotheses have been greatly expanded by the identification of the role of peptides released from the gut and their role in the central control of appetite following food ingestion (Bellisle, Drewnowski, Anderson, Westerterp-Plantenga, & Martin, 2012; El Khoury & Anderson, 2013; Luhovyy, Akhavan, & Anderson, 2007).

In contrast to a large number of studies on the effect of food ingestion on signals reducing appetite and FI, the physiological appetite signals arising from stress, trauma, and disease that lead to the suppression of appetite have received little attention. PA and EX can represent such a stressor under the right conditions. PA and EX affect appetite control, but mechanisms have not been established, thus offering an opportunity for expanding the understanding of the mechanisms of appetite and FI regulation. The definition of PA includes any bodily movement that results in EE and which takes place as part of daily life (Caspersen, Powell, & Christenson, 1985), including sports, household activities, commuting and other activities. EX is defined as a
subset of PA and describes a planned, structured, and repetitive activity to improve or maintain physical fitness levels.
2.3. Physical Activity, Appetite and Body-Weight

Children who engage in regular PA have healthier BWs when compared to their sedentary counterparts (Moore et al., 2003). The maintenance of a healthy BMI and BW in children and adults is associated with habitual PA (Ostojic, Stojanovic, Stojanovic, Maric, & Njaradi, 2011). Children (4 to 11 years of age) in the highest tertile of average daily activity had consistently smaller gains in BMI, triceps, and sum of five skinfolds over 8 years. This beneficial effect of PA was evident for both girls and boys (Moore et al., 2003).

The benefits of PA may arise from its effect on FI and appetite regulation, which is different in habitually active when compared to sedentary individuals. This was first shown by Mayer et al., in 1956. In an observational study that compared physical effort of Bengal workers with their dietary intakes. Their activity ranged from heavy-duty tasks such as lifting and sorting to clerical duties and administrative desk jobs requiring little physical effort. Mayer hypothesized that EIs would closely parallel EE. However, an inverted U-shape was found between EE and FI (Figure 2.1), indicating a relationship between FI and EE only above a certain level of EE. In contrast, sedentary workers had increased levels of EI with low EE, showing a de-regulated FI control likely contributing to subsequent OW/OB (Mayer, Roy, & Mitra, 1956).
Figure 2.1: An adapted version of the original graphic from the article by Mayer et al. (Mayer et al., 1956). The graph shows the relationship between EE and FI in Bengal jute mill workers. Mayer proposed that appetite is accurately regulated in active individuals, but as sedentariness increases, appetite control becomes dysregulated.

More recent studies have confirmed that sedentary individuals generally show a poor homeostatic feedback control of hunger and satiety. They are less able to compensate at a later meal for the energy content of a premeal preload (Kelley & Kelley, 2013; N. A. King, Appleton, Rogers, & Blundell, 1999; Long, Hart, & Morgan, 2002; Van Walleghen, Orr, Gentile, Davy, & Davy, 2007). In addition to merely increasing or decreasing appetite and EI, PA and EX improve the sensitivity of compensatory mechanisms to achieve EB. Energy compensation is defined as the
adjustment of FI or EE provoked by the previous ingestion of a given stimulus (preload), whether a meal, a snack, a beverage, or PA (J. Blundell et al., 2010). Inadequate energy compensation both in short and the long-term has been linked to increased FI and positive EB, leading to a decrease or increase in BW (Jebb et al., 2006; Patel, Bellissimo, Thomas, Hamilton, & Anderson, 2011). EX interventions also improve biomarkers of the metabolic syndrome, such as total cholesterol, low-density lipoprotein cholesterol, insulin, homeostasis model assessment of Insulin Resistance (HOMA-IR), as well as BMI and body fat (BF) (Nascimento et al., 2014) but unlike habitual PA, the effect of EX interventions on BW and BMI is less clear.
2.4. Exercise Interventions, Body-weight, Appetite and Food Intake

EX alters the secretion of gut hormones (Li, Asakawa, Li, Cheng, & Inui, 2011; Schubert et al., 2014; Stensel, 2010), inflammatory (Kasapis & Thompson, 2005) and stress responses (Mastorakos, Pavlatou, Diamanti-Kandarakis, & Chrousos, 2005), which all have been shown to affect appetite and FI. However, the effect of EX interventions on BW and BMI is unclear.

Aerobic EX programs have been found to be highly effective in reducing BW by some studies (Atlantis, Barnes, & Singh, 2006), others found that aerobic-, strength- and resistance EX reduced BF, but without significant improvements in BMI in OW/OB children and adolescents. Children participating in a 6-month physical education program did reduce their BW or BMI status (Thivel et al., 2011).

The lack of effect of EX interventions on BW is potentially induced by a decrease in non-EX related activity (Stubbs, Sepp, Hughes, Johnstone, King, et al., 2002) as well as increased FI (J. E. Blundell et al., 2003). One plausible explanation of why weight change does not occur as expected may be the decrease of EE in non-EX related activities to compensate for the additional expended energy. This has led to the ACTIVITYSTAT hypothesis (T. W. Rowland, 1998). In one study, six healthy NW adult males were assigned to either a sedentary (control) group, a medium intensity EX (383 Kcal/day via two bouts per day for seven days) or high intensity EX (HIEX) groups (766 Kcal/day via three bouts per day for seven days) cycling condition. The authors reported compensation for the additional EE by taking the elevator instead of walking a flight of stairs (Stubbs, Sepp, Hughes, Johnstone, Horgan, et al., 2002). Similarly, a study that followed PA of children by accelerometer measurements throughout the school year in three schools with different PA profiles found that children that were more active at school compensated for
increased EE at a later point in time due to decreases in out of school-related activities (Fremeaux et al., 2011).

Non-EX related EE is not always affected by EX interventions (Baggett et al., 2010; Dale, Corbin, & Dale, 2000; Goodman, Mackett, & Paskins, 2011). For instance, an observational study measured PA with accelerometers in 6,916 American girls and found no evidence of compensation or decrease in non-EX related activities as predicted by the ACTIVISTAT theory (Baggett et al., 2010). Conversely, when researchers decreased the opportunities for PA during the school day, by cancelling physical education classes and restricting children to spend recess in front of computers, they found no compensatory response in preserving a possible set-point of PA through other activities (Dale et al., 2000). As first noted by Mayer (Mayer et al., 1956), FI intake parallels PA above a certain level of activity. Similarly, increased EE has been associated with EX, leading to a decreased loss of BW and BF. Nonetheless, when OW/OB 12-17-year-old adolescents were placed on a 10-week EX-program of cycling, twice a week for 60 minutes, in a controlled environment, mean total FI was not affected. However, considerable inter-individual differences in FI and BW were found. The change in BW and FI fluctuated from a decrease of FI by 1450 kcal and weight-loss of 8 kg in some individuals to an increase in FI by 264 kcal and increase of BW of 9.2 kg (Thivel, Chaput, Adamo, & Goldfield, 2014). Similarly, OW/OB adults showed significant inter-individual differences in response to a 12-week mandated EX-program. Although the study recorded an average weight-change of -3.3 kg, substantial inter-individual differences were recorded from -14.7 kg to +1.7 kg (N. A. King, Hopkins, Caudwell, Stubbs, & Blundell, 2008). The study accounted for large inter-individual variability in weight to compensatory responses in FI (N. A. King et al., 2008; Thivel & Chaput, 2014).
However, OB/OW children and adults may benefit from EX programs more than NW as a result of improvements in appetite control. A 6-week EX program combined with reduced dietary intake showed improvements in hunger and fullness sensations in 38 OB children. The intervention consisted of 1-hour sessions of aerobic EX/day in combination with a caloric restriction (1300 – 3300 kcal/day), based on a child’s basal metabolic rate. The children lost an average 8.4 Kg of BW and showed a more precise appetite control based on altered hunger and fullness scores in response to meals and a fasted state (N. A. King, Hester, & Gately, 2007). In contrast, three weeks of TV watching decreased EE by 99.8 kcal and an increased FI by 250.9 kcal in NW children, resulting in a BW gain of 0.32 Kg per week (Epstein, Paluch, Consalvi, Riordan, & Scholl, 2002). Several studies have shown similar results in adults. For example, one study found that habitual exercisers (>two EX sessions of 40 min or more/week) who consumed either a low (241 kcal) or a high (600 kcal) calorie preload compensated for the calories at a later buffet meal while the non-exercisers did not (Hogenkamp et al., 2013). Variation of responses to EX interventions may also be explained by the different EX modalities, intensities and durations that are used, all of which can potentially affect appetite and FI. For example, EX intensity plays an important role, with higher intensities suppressing appetite and FI while low- and moderate intensities show no effect or even increase EI (Matos et al., 2018).
2.5. The Effect of Acute Exercise on Appetite and Food Intake

Acute moderate EX (40-60% VO\textsubscript{2}max) has been mostly unsuccessful in producing short-term appetite suppression in either children or adults, regardless of body-weight status (WS). Several studies using moderate EX intensities did not detect an appetite and FI response in children (Bellissimo, Thomas, Goode, & Anderson, 2007). Appetite, measured via visual analogue scales (VAS), was increased in 14 healthy weight boys after a 12-minute walking protocol (Bellissimo, Thomas, et al., 2007). A follow-up study tested if acute EX at short and longer durations would increase FI as well as appetite. The study enrolled 14 boys and 15 girls of healthy body weights into four conditions of either two rest or two EX treatments, at the ventilatory threshold \textit{V}\textsubscript{E}T, lasting for either 15 or 45 minutes. In contrast to the previous study, this study found an attenuation of appetite with short duration EX, while the long-duration EX condition resulted in increased appetite ratings when compared to rest. However, the changes in appetite did not translate into changes in FI (Bozinovski et al., 2009). The effect of OW/OB, on post-EX appetite and EI in children, was also examined. 17 OW/OB and 18 NW boys were assigned to an EX condition at their \textit{V}\textsubscript{E}T for 15 minutes, while appetite and FI were compared with resting. Increased appetite at the \textit{V}\textsubscript{E}T was found in both OW/OB and NW children, but a subsequent second study found there was no additional effect of EX at 25% above the \textit{V}\textsubscript{E}T. FI was also not affected by these studies. (Tamam, Bellissimo, Patel, Thomas, & Anderson, 2012). The effect of moderate-intensity EX on FI in 30 NW boys and men was examined in another study. They exercised on a treadmill for 40 minutes at a light to moderate intensity, but no effect of EX on either appetite or FI was found (Hunschede et al., 2015).
In contrast, EX at higher intensities (HIEX) results in an immediate post-exercise reduction in appetite and FI in children and adults. However, WS might be a factor. For example, FI was reduced in OB but not NW study participants after HIEX of 75% VO₂max lasting for 30 minutes and cumulative FI after EX and the next meals throughout the day in OB adolescents (Thivel, Metz, Julien, Morio, & Duche, 2013). Similarly, the same group also investigated the effect an acute HIEX session cycling at 75% VO₂max for 30 min reduced FI at lunch when compared to the control and bed rest session and at a later dinner, when compared with bed rest in OB children. They found that total EI was significantly reduced with HIEX and concluded that the impact of EX or imposed sedentary behaviours on EB in adolescents with OB is not only related to the EX-induced EE but also a decrease in EI (Thivel, Metz, Aucouturier, et al., 2013). Another study in male children and adolescents showed an apparent suppression of appetite after HIEX at 70% VO₂max for 30 min when compared with a rest session; however, there were no differences between NW or OB children (Hunschede, Kubant, Akilen, Thomas, & Anderson, 2017).

Adults also show decreased appetite and EI after HIEX. The reduction of appetite and FI in response to HIEX resulted in a higher energy deficit in OB (Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). The distinctive effect of HIEX compared with more moderate EX was shown by the comparison of resting, moderate, high intensity- and very HIEX. The moderate-intensity session was conducted for 30 min continuously at 60% VO₂max, the high-intensity session for 60s at 100% VO₂max interposed with 240s at 50% VO₂max and the very high-intensity session for 15 s at 170% VO₂max interposed with 60s at 32% VO₂max. FI was decreased only after the very high-intensity session when compared with the moderate and control session, and after the high-intensity session when compared with the resting control (Sim, Wallman, Fairchild, & Guelfi, 2014).
EX mode may also be a factor in determining later appetite and FI as shown by the following two studies. In one study, adolescents EX by cycling at 75% VO\textsubscript{2}max to match the energy expended during three rugby sessions. FI was measured during lunch 30 mins later, at a snack 4h later and at dinner 6.5h later. Only dinner intake reflected the mode of exercise. Dinner intake was increased after aerobic EX (EI: 1185 ± 199 kcal) when compared with rugby (EI: 969 ± 145 kcal) and when rugby was compared with the control session (EI: 777 ± 183 kcal) (Thivel et al., 2015). In a study that compared resistance, aerobic and swimming EX, each with mixed intensities and lasting for 45 minutes, intake was reduced in NW children only by resistance EX. In contrast, in OW/OB children, FI was increased with swimming but remained unchanged with aerobic and resistance EX (Nemet, Arieli, Meckel, & Eliakim, 2010).

Further research is needed to determine duration, amount and type of EX interventions effective for managing BW, BF and BMI in individuals. Similarly, more studies on the effects of EX on the mechanisms of appetite and FI control are needed.
2.6. Mechanisms of Food Intake and Appetite Regulation with High-Intensity Exercise

The majority of research focusing on the mechanisms of appetite and FI regulation has been in response to food ingestion. However, physiological responses to HIEX on regulatory events that might affect appetite and FI regulation are very different. The ingestion of food increases splanchnic blood flow as well as gastric motility (Takala, 1996), while EX has the opposite effect, decreasing splanchnic blood flow and gastric motility (Takala, 1996). During and after food ingestion, increased gastric activation is followed by secretion of GI hormones (G. H. Anderson et al., 2016); however, HIEX stimulates stress responses known to affect appetite and EI.

Anorexia arises from a multitude of signals, which may include integration from appetite, inflammatory and stress responses, as described in Figure 2.2. Appetite control is influenced by an EB framework consisting of tonic (long-term and relatively stable) and episodic (acute varying day-by-day) signals. Episodic signals arise as a reaction to stimuli such as FI and HIEX. These signals are driven by acute excitatory and inhibitory processes.
**Figure 2.** Adapted from Blundell et al. (Hopkins & Blundell, 2016). Appetite control is influenced by an EB framework consisting of tonic (relatively stable) and episodic (varying day-by-day) signals. Episodic signals arise as a reaction to stimuli, such as food intake and HIEX. These signals are driven by acute excitatory (green) and inhibitory processes (red). The effect of EX on appetite control can be understood according to the relative strength of its effects on the tonic and episodic signalling systems. The response of appetite hormones to HIEX is not fully understood. Specific inflammatory biomarkers have strong appetite suppressant properties and have been added to this model of appetite control.

The role of appetite hormones and their effect on appetite and FI in response to HIEX is uncertain, as shown by several studies investigating the effects of HIEX on appetite hormones and FI regulation. At ≥70% VO₂max the appetite-stimulating hormone active ghrelin is decreased (Becker et al., 2012; Broom, Stensel, Bishop, Burns, & Miyashita, 2007; J. A. King, Miyashita, Wasse, & Stensel, 2010; J. A. King et al., 2011; Wasse, Sunderland, King, Batterham, & Stensel,
2012; Wasse, Sunderland, King, Miyashita, & Stensel, 2013), potentially mediating the loss of appetite after HIEX. Furthermore, the appetite-suppressing hormones PYY and GLP-1 are increased by HIEX (Broom, Batterham, King, & Stensel, 2009; Deighton et al., 2013; J. A. King et al., 2010; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Unick et al., 2010; Wasse et al., 2012). In contrast, a comparatively large number of studies, also using HIEX protocols, have shown no response in active ghrelin, PYY or GLP-1 (Balaguera-Cortes, Wallman, Fairchild, & Guelfi, 2011; Broom et al., 2007; Hagobian et al., 2013; Kelly, Guelfi, Wallman, & Fairchild, 2012; J. A. King et al., 2011; Larson-Meyer et al., 2012; Shorten, Wallman, & Guelfi, 2009; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Unick et al., 2010; Wasse et al., 2013). Some studies even showed increased values for active ghrelin (Larson-Meyer et al., 2012) and decreased values for GLP-1 with HIEX (Unick et al., 2010).

In contrast, inflammatory- and stress systems show a consistent response to HIEX, but their effect on appetite has received scant examination. For example, the prototype inflammatory cytokine IL-6 is also a myokine and profoundly affected by HIEX (Kasapis & Thompson, 2005), modulating the immunological and metabolic response to HIEX via its effects on the liver, adipose tissue, hypothalamic-pituitary-adrenal (HPA) axis, leukocytes, and the hypothalamus (Jankord et al., 2010). Several studies show a constant increase with HIEX (Almada et al., 2013; Bruun, Pedersen, Kristensen, & Richelsen, 2002; Bruunsgaard, Ostergaard, Andersen-Ranberg, Jeune, & Pedersen, 2002; Cullen, Thomas, Webb, & Hughes, 2016; Febbraio & Pedersen, 2002; Fischer, 2006; Kaspar et al., 2016; Lyngso, Simonsen, & Bulow, 2002). Although less consistent when compared to IL-6, other studies have also shown increased inflammatory biomarkers CRP after strenuous HIEX (Drenth, Krebbers, Bijzet, & van der Meer, 1998; Liesen, Dufaux, & Hollmann,
1977). TNF-α, on the other hand, has not shown to be affected by HIEX (Kasapis & Thompson, 2005), but suppresses appetite within cachexia (Espat, Copeland, & Moldawer, 1994). Furthermore, cortisol a primary stress biomarker is consistently increased with HIEX (Hayes, Grace, Baker, & Sculthorpe, 2015). Although inflammatory and stress biomarkers show a more consistent response to HIEX, and there is indirect evidence that they may have appetite mediating properties, their role in appetite regulation with HIEX has not been reported. The following paragraphs provide a more detailed examination of the role of peptide hormones, stress and inflammatory biomarkers in appetite control.

2.6.1. Gastrointestinal- and Adiposity derived Appetite Signals

Key regulators of FI are the gastrointestinal tract and the brain which provide bi-directional communication via hormones and metabolites. The critical role of the hypothalamus and the caudal brainstem in with several hormonal and neural mechanisms by which the brain informs itself about nutrient storage and, in turn, generates behavioural, autonomic, and endocrine output that influence appetite and FI regulation, have been identified in recent years (Lenard & Berthoud, 2008). With the discovery of the adipokine leptin, an EB and BW regulation model were introduced in which caloric intake, nutrient partitioning, fuel utilization, and energy storage are controlled by neuroendocrine circuits of the hypothalamus and the hindbrain. According to this model, energy homeostasis is maintained by a constant flow of information from the gastrointestinal (GI) system and the adipose tissue to specific AgRP/NPY and pro-opiomelanocortin (POMC) neurons in the arcuate nucleus (ARC) of the hypothalamus, thereby providing feedback on the current energy status and requirements. In return, neuropeptides and
neurotransmitters in the CNS are released and respond via neurons of the autonomic nervous system and the endocrine system to the target organs. As a result, energy homeostasis is constantly monitored and dynamically regulated by compensation mechanisms of multiple mechanisms.

Appetite hormones are major components in mechanisms leading to the regulation of energy homeostasis. These hormones are generally arising as secretions from enteroendocrine cells in the stomach, pancreas, small intestine, adipocytes and other sites that control numerous functions of the digestive organs. They have many additional functions in metabolic, behavioural, cardiovascular, reproductive, and immunologic processes (Kojima & Kangawa, 2008). Most prominent, appetite hormones include leptin, ghrelin, GLP-1, PYY, which are reviewed in the following paragraphs.

2.6.1.1. Leptin

Leptin is a hormone predominately secreted by adipose cells, regulating body fat and suppressing appetite. Like other appetite-regulating hormones, leptin acts on the ARC of the hypothalamus regulating energy homeostasis and conveys an “adiposity negative feedback signal” communicating the amount of fuel stored in fat to the hypothalamus. In NW individuals, circulating leptin reduces appetite and FI (Klok, Jakobsdottir, & Drent, 2007); however, in OW/OB populations leptin is found in higher concentrations (Crujeiras et al., 2015) but does not reduce EI, suggesting a resistance to leptin signalling similar to that of insulin in Type 2 diabetes (Considine et al., 1996; Myers et al., 2008). Most acute studies do not show a direct effect of EX
on leptin action (Elias et al., 2000), some studies show a decrease of leptin with EX which can be attributed to diurnal changes in leptin rather than a direct effect of EX (Kraemer et al., 1999). In humans, EX interventions do not affect circulating levels of leptin in NW human subjects but decrease leptin levels in OW/OB individuals (Sari, Balci, Balci, & Karayalcin, 2007). In theory, a decrease in circulating leptin would favour higher appetite/FI after an EX intervention and support weight gain. However, Wistar rats showed an increased leptin and insulin sensitivity after an EX intervention for 6h/day for five days at moderate to severe intensities when compared to a resting control group. Infusion with leptin and insulin suppressed FI in the EX group but not the sedentary group, suggesting improvements in appetite control by altering leptin and insulin sensitivity rather than secretion (Flores et al., 2006). Furthermore, other hormones such as insulin, cortisol, catecholamines may affect leptin production, and it is, therefore, essential to investigate leptin secretion with EX (Kraemer, Chu, & Castracane, 2002).

2.6.1.2. Ghrelin

Ghrelin is a 28 amino acid peptide hormone, which is released by endocrine cells in the stomach, stimulating appetite (Kojima & Kangawa, 2005). Ghrelin is classified as an episodic appetite signal, decreasing post-prandially and in proportion to the caloric load and increasing in the fasted state (Tschop et al., 2001). Ghrelin cells are mainly found in the mucosa of the stomach (Ariyasu et al., 2001), and its secretion is down-regulated in response to carbohydrate ingestion (Shiiya et al., 2002). Exogenous ghrelin administration increases FI in both humans (Wren, Seal, et al., 2001) and rodents (Wren, Small, et al., 2001). Ghrelin itself only exerts its effects in its acylated (active) form, which can cross the blood-brain barrier exerting its effects in the ARC
stimulating NPY/AgRP neurons subsequently increasing appetite (H. Y. Chen et al., 2004). EX interventions result in increased levels of ghrelin and are negatively correlated with BW (Cummings et al., 2002), possibly making weight loss more difficult. Excessive weight gain may also impair ghrelin’s role in appetite control, as shown by lower circulating ghrelin concentrations in OB/OW individuals who are also showing a compromised control of FI when compared to NW (English, Ghatei, Malik, Bloom, & Wilding, 2002). Another EX intervention has shown a reduction of BW and fasting insulin concentrations as well as an increase in fasting acylated ghrelin levels and hunger sensations, with a tendency towards decreased acylated ghrelin and increased satiety levels post-prandially, in response to the 12-week HIEX program five times/week (J. A. King et al., 2010).

In the short-term, active ghrelin has been found to be decreased for 3h after EX for 60 min at 72% VO₂max, which was also associated with decreased hunger sensations (Broom et al., 2007). Another study also showed that HIEX at 65%, and 72% VO₂max decreased acylated ghrelin for 60 minutes post-EX (Broom et al., 2009). Studies investigating the effect of EX at ≥70% VO₂max consistently found a decrease in the appetite-stimulating hormone active ghrelin (Becker et al., 2012; Broom et al., 2007; J. A. King et al., 2010; J. A. King et al., 2011; Wasse et al., 2012; Wasse et al., 2013). Acute HIEX has been shown to lower active ghrelin levels, possibly contributing to the suppression of appetite or FI post-EX (Sim et al., 2014).
2.6.1.3. Glucagon-Like-Peptide-1

GLP-1 is an incretin and neuropeptide suppressing appetite. It is also a derivative from post-translational modifications of the pre-proglucagon gene, released from the L-cells in the intestines. GLP-1 acts to stimulate insulin production and inhibit glucagon secretion. Both central and peripheral injection of GLP-1 has been shown to inhibit FI in both, humans (Verdich et al., 2001) and animals (Turton et al., 1996), potentially mediated by carbohydrate and fat ingestion (Harrold, Dovey, Blundell, & Halford, 2012). In a 12-week intervention study, GLP-1 showed post-EX increases in GLP-1 (Ueda et al., 2013). With acute HIEX, the role of GLP-1 is uncertain. GLP-1 was increased following EX for 60 min at 50% VO$_2$max with no differences in hormone responses between NW and OW/OB study participants (Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). The same group also investigated appetite hormone responses to EX for 30 min at 50% and 75% VO$_2$max. The study found increases in GLP-1 at both intensities when compared with rest (Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). However, other studies have shown an increase of GLP-1 after HIEX (Deighton et al., 2013; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). Furthermore, the mechanisms of how HIEX can stimulate GLP-1 secretion remains unknown.

2.6.1.4. Peptide-YY

PYY is a hormone that is produced in endocrine L-cells which are located in the small intestine and suppresses appetite. PYY belongs to the NPY family (Cummings & Overduin, 2007) and inhibits NPY neurons while stimulating anorexic POMC neurons, thus suppressing appetite and FI (Batterham et al., 2002; Cummings & Overduin, 2007). Peripheral infusion with PYY has been shown to suppress FI in humans (Batterham et al., 2002) and animals (Pittner et al., 2004).
GLP-1 and PYY are both co-released due to their similar production sites and display similar effects on appetite suppression (Neary et al., 2005). A 12-week intervention study resulted in increased levels of the fasted post-EX tendency for an increase in PYY (Ueda et al., 2013). Acutely, PYY has been shown to be increased with HIEX at 75% VO2max but not 50% VO2max, indicating an EX-intensity dependent effect (Deighton et al., 2013; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). However, other studies have shown no effect of HIEX on PYY (Larson-Meyer et al., 2012), making it not a reliable biomarker of appetite and FI with HIEX.

Appetite-regulating hormones show consistent responses to FI but not EX. Active Ghrelin seems to be an appetite biomarker that is more consistently affected by EX when compared to GLP-1 and PYY. Various other appetite-regulating hormones are also involved in appetite control but excluded from this review. GI appetite signals such as a pancreatic polypeptide (PP), cholecystokinin (CCK) and amylin have not been well documented in response to PA and EX.

2.6.2. Appetite Regulation and the Inflammatory and Stress Systems

Acute inflammation has a strong anorexic effect. Over decades, the link between inflammation and suppression of appetite has been well established (Gautron & Laye, 2009). Inflammation is a multifactorial process involving immune cells, blood vessels, and other molecular mediators. Inflammation and the simultaneous decrease of appetite have also been proposed to be beneficial for survival in the presence of infection or other diseases (Wing & Young, 1980). This may occur due to energy repartitioning in support of immune responses by reducing activities such as food seeking and digestion (Exton, 1997; Hart, 1988) as well as by a decrease in micronutrient availability needed for bacterial proliferation (Weinberg, 1984).
However, high levels of chronic inflammation are detrimental to survival, if chronically overused, it may result in cachexia, long-term suppression of appetite and FI in humans. In humans, cachexia is often observed with the chronic obstructive pulmonary disease, chronic kidney disease or cancer, and accompanied by severe loss of BW and increase in mortality (von Haehling & Anker, 2014). There is a considerable amount of evidence suggesting a role of inflammatory cytokines in cachexia and anorexia in humans (K. T. Murphy, Cobani, Ryall, Ibebunjo, & Lynch, 2011; Zhou et al., 2010), the most prominent ones being C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), and Interleukin-6 (IL-6). Each of the cytokines has a different transducing system, and the anorectic effect is dependent on the type of cytokine, dosage, and duration as well as the route of administration. Patients with HIV and cancer often display chronic and extremely high levels of inflammation and drastic weight-loss, accompanied by increased mortality, and a general loss of appetite (Laviano, Meguid, & Rossi-Fanelli, 2003; Tisdale, 2009). In these patients, non-steroidal anti-inflammatories such as indomethacin and ibuprofen have been tested and showed a reduction in inflammatory and stress biomarkers IL-6 and cortisol (Vaughan, Martin, & Lewandowski, 2013). One trial also demonstrated significant weight (12 weeks: Δ 5.1 kg; p< 0.001) and appetite (EORTC QLQ-C30) improvements from baseline (p< 0.05), in patients treated with ibuprofen (McMillan et al., 1999).

In contrast, elevated, albeit to a much lesser degree, low grade inflammation and altered appetite hormones are found in the presence of the metabolic syndrome and obesity (Chedraui et al., 2014; Indulekha, Surendar, & Mohan, 2011; Weiss, Arnesen, & Seljeflot, 2013), arising from excess FI and the accumulation of adipose tissue. Therefore, it appears counterintuitive that the acute inflammation related to many diseases suppresses appetite, but a link is plausible.
Inflammation can be categorized as either acute or chronic. With chronic inflammation, the cytokines, IL-6, TNF-α and CRP, are upregulated by and expressed due to increased macrophages in adipose tissue (Hotamisligil, Shargill, & Spiegelman, 1993; Jung & Choi, 2014; Ouchi, Parker, Lugus, & Walsh, 2011), causing low-grade systemic inflammation and subsequent insulin resistance (Kern, Ranganathan, Li, Wood, & Ranganathan, 2001). In the development of obesity and even within several days of ingesting a high-fat and calorie-dense diet, saturated fatty acids from the periphery cross the BBB and induce an inflammatory response in hypothalamic neurons (Thaler et al., 2012), indicating the onset of chronic hypothalamic inflammation and dysfunctional appetite regulation. It has recently been established that constitutive activation of the pro-inflammatory pathways in AgRP neurons increases impulsive firing in these cells, along with neuronal and systemic leptin resistance, resulting in hyperphagia, increased weight gain and adiposity (Tsaousidou et al., 2014). In summary, inflammation in the brain impairs appetite regulation, energy homeostasis, induces hyperphagia and a dysregulated appetite control, most likely due to decreased insulin and leptin sensitivity (Timper et al., 2017) leading to excess FI over time.

Acute infections or trauma such as HIEX increase inflammation and are linked to a suppression of appetite and FI in animals and humans (Gautron & Laye, 2009). Furthermore, inflammation-induced anorexia has been hypothesized to be part of host defence, instead of a simple by-product (Murray, Murray, & Murray, 1978). In one experiment, an infection was induced in mice by virulent bacteria. Mortality and macrophage activity increased when mice were force-fed and compared to mice who were allowed to maintain an anorexic state (Wing & Young, 1980). Anorexia may, therefore, be an essential part of an acute inflammatory response.
This acute phase response is not well understood in humans, and most evidence comes from animal studies where inflammation is induced exogenously (Boelen, Platvoet-ter Schiphorst, Bakker, & Wiersinga, 1995) (Boelen et al., 1995). Those studies showed that peripheral administration of lipopolysaccharide (LPS) could reduce meal frequency (Langhans, Savoldelli, & Weingarten, 1993) and induce an aversion for certain foods (Langhans, Harlacher, Balkowski, & Scharrer, 1990; Tazi, Dantzer, Crestani, & Le Moal, 1988). Although appetite cannot be precisely determined in laboratory animals, it is well established that the reduction in appetite accounts for the total reduction in FI with acute inflammatory responses. Various cytokines have been involved with the inflammatory reduction in appetite and FI with the most prominent ones such as CRP, TNF-α, and IL-6. Each of the cytokines has a different transducing system, and the anorectic effect is dependent on the type of cytokine, dosage, and duration as well as the route of administration.

Several mechanisms have been proposed for how cytokines can affect appetite. First, cytokines act in similar ways as peptides and control feeding by modulating the hypothalamic environment. Cytokines are transported across the blood-brain barrier where they interact with the luminal surface of brain endothelial cells to release substances that affect appetite (Banks, 2001). Data has shown that cytokines can inhibit appetite directly by stimulating hypothalamic neurons sensitive to glucose in the lateral hypothalamic area. This has been shown for cytokines such as IL-6, IL-1β and TNF-α (Jankord et al., 2010; Plata-Salaman, 1996), which have receptors in the hypothalamic area of the brain which regulate appetite and FI (Hellerstein, Meydani, Meydani, Wu, & Dinarello, 1989). They can also exhibit their anorectic effects on neuropeptide systems such as corticotropin-releasing factor or NPY (Laviano et al., 1996; Plata-Salaman, 1991).
Second, cytokines are thought to mediate GI activities including a decrease gastric motility and gastric emptying. Intraperitoneal injections of human TNF-α receptor antagonist improved FI in tumour-bearing rats (Torelli et al., 1999). Third, cytokines inhibit appetite indirectly through other endogenously released substances via the release of putative physiological anorectic and orexigenic signals such as glucagon, insulin, ghrelin, and leptin (Plata-Salaman, 1998).

### 2.6.2.1. C-reactive Protein

C-reactive protein is an acute-phase protein that is found in blood plasma and produced by the liver. It parallels in response to inflammation to bind to the surface of dead or dying cells in order to activate the complement system and help to clear out pathogens from an organism. CRP is often used to identify inflammation within OB and levels of CRP are reduced after weight loss (Tchernof, Nolan, Sites, Ades, & Poehlman, 2002). In the long-term, CRP has been hypothesized to block the action of leptin within OB and disrupt the hormones function to terminate eating (K. Chen et al., 2006) which would lead to a lack of appetite control and further weight gain. However, these findings are limited to animal studies. In humans, numerous studies have shown an association of CRP and appetite with cancer or undergoing hemodialysis (Caliskan et al., 2012). In these patients, higher CRP levels are associated with lower appetite scores leading to cachexia (Kalantar-Zadeh, Block, McAllister, Humphreys, & Kopple, 2004). On the short-term, there is no information regarding the effects of CRP levels and appetite in NW or OB individuals.

### 2.6.2.2. Interleukin-6

Interleukin-6 is a pro-inflammatory cytokine as well as an anti-inflammatory myokine. In response to infection or trauma, IL-6 is produced by the T-cells and macrophages, and its anti-inflammatory role is mediated through its inhibitory effects on TNF-α, IL-1, and activation of IL-
1ra and IL-10. With EX, it is significantly elevated and precedes the appearance of other cytokines in the circulation. During EX, it is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery (Petersen & Pedersen, 2005). If IL-6 is administered to laboratory animals, feeding is inhibited (Langhans & Hrupka, 1999) and an increase in the inflammatory biomarkers IL-6, TNF-α and CRP have been associated with a suppression of appetite (Kasapis & Thompson, 2005; Morley et al., 2006). In rodents, elevated inflammation associated with suppression of appetite when induced by liposaccharide (LPS) injection (Boelen et al., 1995) or by direct exogenous administration of inflammatory cytokines such as IL-6 (K. Wallenius et al., 2002). Furthermore, IL-6 deficient mice develop mature-onset OB (V. Wallenius et al., 2002) and display greater EIs when compared with wild-type mice (Chida, Osaka, Hashimoto, & Iwakura, 2006). In humans, an inverse association between inflammation and suppression of appetite and FI is also found during cachexia (Gautron & Laye, 2009; Solheim, Fearon, Blum, & Kaasa, 2013), a condition characterized by a rapid loss of appetite and BW (Gautron & Laye, 2009). Interleukin-6 is increased during and after EX, especially with higher intensities (Fischer, 2006). Children also show increases in IL-6 after 40 min of vigorous EX (Scheett, Mills, Ziegler, Stoppani, & Cooper, 1999). IL-6, as well as TNF-α, have been hypothesized to be a major drivers in the loss of appetite (Morley et al., 2006). Following HIEX in OW/OB adults, a more pronounced increase in the inflammatory biomarkers IL-6 and TNF-α is found, when compared with NW individuals (Christiansen et al., 2013). OB adolescents also display reduced FI after intense EX when compared to NW individuals (Thivel et al., 2012). However, a relationship of HIEX and appetite/FI and inflammation has never been investigated in humans.
2.6.2.3. Tumor Necrosis Factor Alpha

TNF-α is also a cytokine, produced by activated macrophages. Its primary function is the regulation of immune cells, but it is also involved in thermoregulation (Baxter, Kuo, Jupp, Vandenabeele, & MacEwan, 1999), apoptosis (Leon, White, & Kluger, 1998), human cachexia (Espat et al., 1994) and glucose metabolism (Ciaraldi, Carter, Mudaliar, Kern, & Henry, 1998). TNF-α is potentially increased by HIEX (Bernecker et al., 2013), but in contrast to IL-6 this relationship is not well established.

TNF-α is heavily involved in appetite control, acute and chronic administration of TNF-α reduces FI and reproduces effects similar to cancer cachexia (Gelin et al., 1991; Matthys & Billiau, 1997; Noguchi, Yoshikawa, Matsumoto, Svaninger, & Gelin, 1996). Despite the mechanistic explanation, the role of TNF-α in human cancer cachexia is unclear.

2.6.3. Other Appetite Related Biomarkers Affecting Appetite and Food Intake with High-Intensity Exercise

2.6.3.1. Cortisol

Stress and its primary biomarker Cortisol has long been implicated in the regulation of appetite. It remains unknown if and under which circumstances cortisol triggers under-/overeating in response to acute stress reactions. Some studies suggest that there is no direct effect of cortisol on stress-related eating behaviours and instead, other factors such as leptin or specific cytokines, triggered in the presence of cortisol, may have a more direct effect on eating after a stressful situation (Sapolsky, 1998). In addition, catecholamines (adrenaline and noradrenaline), released from the adrenal glands in response to stress, may also affect FI through a slowed gastric
emptying (Torres & Nowson, 2007). The injection of cortisol has been shown to decrease FI, and the anorexic effect of cortisol has been hypothesized to act via two possible pathways: 1) The decrease of ghrelin receptor expression in the hypothalamus and ghrelin secretion and 2) a decreased NPY expression (Janzen, Duncan, & Riley, 2012). However, exact mechanisms of how cortisol can affect appetite need to be elucidated.

2.6.3.2. Blood Glucose and Insulin

Blood glucose and insulin have long been implicated in the regulation of appetite, starting with Jean Mayer’s glucostatic theory of appetite regulation in the 1950s. He hypothesized that peripheral and central glucose receptors sense fluctuations in arteriovenous glucose availability and signal changes in appetite and eating behaviour (Mayer, 1953). His theory is supported by the fact that a decrease in blood glucose concentrations was associated with increased sensations of hunger and meal initiation in humans (Grossman & Stein, 1948; Melanson, Westerterp-Plantenga, Saris, Smith, & Campfield, 1999; Thompson & Campbell, 1977) and increased FI in laboratory animals (Grossman, Cummins, & Ivy, 1947; Houpt, 1974; Houpt & Hance, 1971; N. Rowland, 1978). Conversely, treatments that increase blood glucose also associated with increased feelings of satiation and satiety in humans (G. H. Anderson & Woodend, 2003; Furchner-Evanson, Petrisko, Howarth, Nemoseck, & Kern, 2010; Howarth, Petrisko, Furchner-Evanson, Nemoseck, & Kern, 2010) and reduced FI in experimental animals (Geary & Smith, 1982; Martin & Novin, 1977; Vanderweele, Geiselman, & Novin, 1979). Insulin on the other was initially thought to not affect appetite directly but to induce a hunger response by increasing hypoglycemia (Lotter & Woods, 1977). However, arising from the observation that
insulin crosses the blood-brain barrier (BBB) and exerts its effects on via insulin receptors in the brain, a more direct role for it in regulating FI has developed. (Plum, Schubert, & Bruning, 2005). Centrally administered insulin has been shown to reduce FI and cause a reduction of BW in animals (M. W. Schwartz, Figlewicz, Baskin, Woods, & Porte, 1992; Woods & Seeley, 2001), and conversely, when insulin-antibodies are administered animals overeat (McGowan, Andrews, & Grossman, 1992). These results have lead to the proposal that insulin in the brain plays a role in long-term regulation of FI, and does not regulate FI by immediate reflection of its concentration in blood in response to food ingestion (G. H. Anderson, Luhovyy, Akhavan, & Panahi, 2011).
2.7. Summary and Research Rationale

EX represents a plausible means to improve the sensitivity of the appetite control system, but the mechanisms by which the benefits of EX arise have received little attention in adults and almost none in children.

Inflammatory cytokines have strong appetite suppressing properties, similar to appetite hormones. However, their secretion is dependent on a different type of stimulus, such as an infection or trauma versus the ingestion of food. HIEX represents a form of stress that causes an increase in inflammatory cytokines. Furthermore, the muscle acts as an endocrine organ producing IL-6 after bouts of HIEX (Petersen & Pedersen, 2005). Therefore, it seems logical for inflammatory cytokines to be involved in the suppression of appetite and FI after HIEX.

Therefore, the objective of this research was to begin elucidating an understanding of the role of inflammation in mechanisms by which HIEX affects appetite and FI in male children and adolescents.
Chapter 3
HYPOTHESIS AND OBJECTIVES

3.1. General Hypothesis and Objective

Hypothesis

- Select inflammatory biomarkers respond to HIEX, but not macronutrient preloads and subsequently be associated with a decrease short-term appetite and FI independent of appetite hormones in NW and OB boys.

Objective

- To identify the effects of HIEX and macronutrient consumption on appetite, FI, and selective appetite- and inflammatory-/stress biomarkers in NW and OB children.
3.2. Specific Rationales, Hypothesis, and Objectives

**Chapter 4:** DECREASED APPETITE FOLLOWING HIGH-INTENSITY EXERCISE CORRELATES WITH INCREASED PLASMA IL-6 IN NORMAL-WEIGHT AND OVERWEIGHT/OBESE BOYS.

**Rationale:**

- The effects of HIEX induced inflammatory- and stress biomarkers on appetite control and Body-Weight has not been reported in children or adults.

**Hypothesis:**

- HIEX induced suppression of appetite associates with inflammatory- and stress-, but not appetite hormone responses in OW, OB and NW boys

**Objective:**

- The objective of this study was to describe the effects of acute HIEX at 70% VO_{2}peak on post-exercise appetite and selective biomarkers of inflammation, stress, and appetite regulatory hormones in NW and OW/OB boys.
Chapter 5: THE EFFECTS OF IL-6 FOLLOWING HIGH-INTENSITY EXERCISE AND IBUPROFEN CONSUMPTION ON APPETITE AND FOOD INTAKE IN NORMAL-WEIGHT BOYS.

Rationale:

- The role of IL-6 in exercise-induced anorexia in normal-weight children is unknown.

Hypothesis:

- IL-6 response in response to HIEX (75% VO₂peak) and Ibuprofen, is associated with the suppression of appetite and FI in NW boys.

Objective:

- To investigate the effect of HIEX and Ibuprofen on plasma levels of IL-6 and other selective biomarkers of inflammation and appetite on FI and subjective ratings of appetite in NW boys.
Chapter 6: ACUTE RESPONSE IN INFLAMMATORY BIOMARKERS AND APPETITE SUPPRESSION AFTER GLUCOSE AND PROTEIN BEVERAGES IN MALE ADOLESCENTS.

Rationale:

- The short-term response of inflammatory biomarkers and their role in satiety and appetite after glucose or protein intake has not been reported.

Hypothesis:

- IL-6 and TNF-α respond acutely to glucose, and protein macronutrient preloads in male adolescents and play a role in appetite and FI independent of appetite hormones.

Objective:

- To describe the effects of glucose and protein preloads on biomarkers of inflammation, appetite hormones and their subsequent effect on appetite and FI regulation in male adolescents.
Chapter 4
DECREASED APPETITE FOLLOWING HIGH-INTENSITY EXERCISE CORRELATES WITH INCREASED PLASMA IL-6 IN NORMAL-WEIGHT AND OVERWEIGHT/OBESE BOYS.

The following chapter is a reproduction of a manuscript that has been published in Current Developments in Nutrition Vol. 1, Issue 5; 1 May 2017.
Title: Decreased Appetite Following High-Intensity Exercise Correlates with Increased Plasma IL-6 in Normal-weight and Overweight/Obese boys.

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Abstract:

Background: High-intensity exercise (HIEX) suppresses appetite in adults and is thought to be mediated by appetite-regulating hormones. However, the effects of HIEX induced inflammatory- and stress biomarkers on appetite control and body-weight (BW) has not been reported in children or adults.

Hypothesis: HIEX induced suppression of appetite associates with inflammatory- and stress-, but not appetite hormone responses in overweight (OW), obese (OB) and normal-weight (NW) boys.

Objective: The objective of this study was to describe the effects of acute HIEX at 70% VO$_2$ peak on post-exercise appetite and selective biomarkers of inflammation, stress, and appetite regulatory hormones in NW and OW/OB boys.

Design: NW (n=11) and OW/OB (n=11) boys (aged 10-18y) were randomly assigned in a crossover design to rest or HIEX. The HIEX protocol consisted of three 10min bouts of HIEX at 70% VO2peak at 60-70RPM with 1:30min active rest interposed. Visual analog scale appetite ratings and plasma biomarkers of appetite, inflammation, stress, and glucose control were measured following HIEX or rest.

Results: Appetite increased from baseline to 70min (p<0.001) but was lower after HIEX (p=0.04) with no difference between BW groups. HIEX also resulted in lower active ghrelin (p<0.001) and increased interleukin-6 (p<0.001), tumour-necrosis-factor-α (P<0.001) and cortisol (P<0.001) levels, independent of BW. It increased blood glucose (P=0.002) and insulin (P=0.028) concentrations in NW but not OW/OB boys. Leptin, glucagon-like-peptide-1, peptide-YY, C-reactive protein and cortisol were not affected by HIEX over time. An inverse correlation was found between Interleukin-6 and appetite (r=−0.379; P=0.012) but not any other biomarkers.

Conclusion: In conclusion, HIEX resulted in reduced appetite that correlated with an increase in Interleukin-6 in both NW and OW/OB boys. However, while a role for Interleukin-6 in the response can be suggested, the suppression of appetite was potentially mediated by the decrease in active ghrelin and/or increase in cortisol.
4.1. Introduction

The short-term effect of exercise on energy intake (EI) regulation is dependent on intensity, modality (Panissa et al., 2016) and duration (Bozinovski et al., 2009). Acute low intensities produce no or only moderate effects on appetite (N. A. King et al., 1994; Thompson et al., 1988), while exercise at high intensities above 70% maximum oxygen consumption (VO$_2$peak) suppresses appetite (J. E. Blundell & King, 2000; Sim, Wallman, Fairchild, & Guelfi, 2013) and EI (N. A. King et al., 1994; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009) in healthy adults and obese (OB) men (Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). High-intensity exercise (HIEX), defined by the American College of Sports Medicine as exercise intensities equal to or above 70% VO$_2$peak (Pescatello & American College of Sports Medicine., 2014) is not to be confused with high-intensity interval or intermitted training.

The role of appetite hormones in the reduced appetite following HIEX is uncertain. Appetite hormones are well known to play critical roles in nutrient signalling and subsequently controlling appetite (Ahima & Antwi, 2008). The ingestion of food stimulates the release of appetite hormones, such as ghrelin, peptide-YY (PYY) and glucagon-like-peptide-1 (GLP-1). In one study, an acute bout of HIEX lowered active ghrelin and increased PYY as well as glucagon-like-peptide 1 (GLP-1) (Deighton et al., 2013; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). In contrast, HIEX has been reported to increase active ghrelin (Larson-Meyer et al., 2012), decrease GLP-1 (Unick et al., 2010) and have no effect on PYY (Larson-Meyer et al., 2012). Thus, as concluded in a meta-analysis of current studies, acute bouts of exercise may have only slight to moderate effects on appetite-regulating hormones (Schubert et al., 2014).
Acute HIEX elicits many physiologic responses other than appetite hormones, such as the immune- and stress system (Brown, Davison, McClean, & Murphy, 2015; Hamer, Taylor, & Steptoe, 2006), that may account for a post-exercise appetite suppression. Similar to appetite hormones such as active ghrelin, GLP-1, PYY and Leptin, inflammatory cytokines such as Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α) cross the blood-brain barrier and directly interact with the luminal surface of brain endothelial cells to release substances that can affect appetite (Banks, 2001). For example, IL-6 and TNF-α stimulate expression of proopiomelanocortin (POMC) (Katahira, Iwasaki, Aoki, Oiso, & Saito, 1998), a neuropeptide that suppresses appetite in the hypothalamus. Hypothalamic IL-6 receptors are also present in the arcuate-, dorsomedial-, ventromedial-, lateral-, paraventricular- and supraoptic nucleus, exerting their effect on appetite (Benrick et al., 2009; Ropelle et al., 2010; Saper, Chou, & Elmquist, 2002; Shizuya et al., 1997).

An increase in the inflammatory biomarkers IL-6, TNF-α and CRP have been associated with a suppression of appetite. In rodents, elevated inflammation associated with suppression of appetite when induced by liposaccharide (LPS) injection or by direct exogenous administration of inflammatory cytokines such as IL-6 and TNF-α. Furthermore, IL-6 deficient mice develop mature-onset obesity (V. Wallenius et al., 2002) and display greater EIs when compared with wild-type mice (Chida et al., 2006). In humans, an inverse association between inflammation and a suppression of appetite/EI is also found during cachexia (Gautron & Laye, 2009; Solheim et al., 2013), a condition characterized by a rapid loss of appetite and body-weight (BW) (Gautron & Laye, 2009), where IL-6, as well as TNF-α, have been hypothesized to be major driver in the loss of appetite (Morley et al., 2006). Following HIEX in OW/OB adults, a more pronounced increase
in the inflammatory biomarkers IL-6 and TNF-α is found, when compared with normal-weight (NW) individuals (Christiansen et al., 2013). OB adolescents also display reduced EI after intensive exercise when compared to NW individuals (Thivel et al., 2012).

Because IL-6 is derived from muscle and increases in blood with HIEX, it is a possible cause of the suppression of appetite. IL-6 is a mediator of muscle protein biosynthesis (Raj et al., 2008), thermoregulation (De Jongh et al., 2003), EE (Wernstedt et al., 2006) and the regulation of BW (V. Wallenius et al., 2002) and in contrast to cachexia, TNF-α is not increased by exercise (Croft et al., 2009). However, only one study has reported that an increase in IL-6 with HIEX is associated with suppression of appetite and EI (Almada et al., 2013). In this study of twins, one twin was exposed to 45 minutes of submaximal exercise intensity near the anaerobic threshold and 7 minutes of 90% of VO\textsubscript{2}peak. Plasma IL-6 concentrations increased and post-exercise EI decreased. However, there has been no report in either adults or children of HIEX induced suppression of appetite with its concurrent effects on appetite hormones, stress, and inflammatory responses.

Therefore, we hypothesized that acute suppression of appetite after HIEX (at 70% VO\textsubscript{2}peak) is associated with IL-6, but not appetite hormone response in both OW/OB and NW children and adolescents. The objective of this study was to describe the effects of acute HIEX at 70% VO\textsubscript{2}peak on post-exercise appetite and selective biomarkers of inflammation, stress, and appetite regulatory hormones in NW and OW/OB boys.
4.2. Materials and Methods

4.2.1. Participants

11 NW (BMI for age percentile: 15\textsuperscript{th}-85\textsuperscript{th}) and 11 OW/OB (BMI for age percentile: > 90\textsuperscript{th}) boys, aged 10-18 years (Flegal, Wei, & Ogden, 2002), were recruited through local advertisements. The participant characteristics are shown in Table 4.1. Sample size was determined from previous studies in our lab. Eligibility for the study was determined via a telephone screening questionnaire. Boys who answered “yes” to one of the questions of the physical activity readiness questionnaire, displayed fear of venipuncture, dieters, have been diagnosed with diabetes or other metabolic diseases, and those scoring ≥11 on an Eating Habit Questionnaire were excluded from the study. All experimental procedures have been approved by the University of Toronto Health Sciences Research Ethics Board, and informed consent was obtained from all adult participants, parents of the children as well as assent from the children themselves. Four participants did not complete the study, due to difficulties accessing the veins or a mild form of vasovagal syncope, likely due to the IV-catheter insertion.

4.2.2. Subject Assessment

For the screening session, parents and their children attended the University of Toronto Goldring centre for High-Performance Sport during the week or on weekends. The screening session was conducted to determine physical fitness, anthropometric measurements, habitual physical activity levels, and pubertal status using Tanner stages (W. A. Marshall & Tanner, 1970). Physical fitness was determined via the measurement of VO\textsubscript{2}peak and the ventilatory threshold (V\textsubscript{ET}), using a continuous incremental cycling protocol on a Kettler RE7 recumbent bicycle.
(Kettler, Ense-Parsit, NRW, Germany). Participants cycled for 3 min at 25 Watts, then the intensity was increased every minute by 15 watts for boys weighing < 60 kg and 20 Watts for boys > 60 kg. The \( V_eT \) was assessed using the ventilatory equivalent method (Caiozzo et al., 1982) and VO\(_2\)peak determined using the highest six consecutive breaths (Hunschede et al., 2015). Body mass index (BMI) was used to identify participants with healthy body weight (15\(^{th}\)-85\(^{th}\) BMI for age percentile) or OW/OB (90\(^{th}\)-100\(^{th}\) BMI for age percentile), according to the Center for Disease Control and Prevention (2000) growth charts (Flegal et al., 2002). Bioelectrical impedance analysis was used to estimate body fat mass and fat-free mass (RJL Systems BIA, 101Q) based on the Horlick equation (Horlick et al., 2002).

### 4.2.3. Study design and Experimental Protocol

The study employed a three-level mixed factorial (ACTIVITY × WEIGHT STATUS × TIME) repeated-measures randomized design. All participants completed two test sessions: (i) exercise, 30 min of HIEX at 70% VO\(_2\)peak or (ii) no-exercise, 30 min of rest. The randomization schedule was generated using the SAS PROC PLAN procedure (SAS Institute v. 9.3, Cary, NC, USA). Experimental sessions were conducted at the University of Toronto Athletic centre on weekends between 9 and 10 am after a 12-h overnight fast. Children were asked to refrain from exercise at least 24-h before the experimental session and parents were asked to encourage their children to drink water up to 1-h before the scheduled session, to refrain from physical activity, and to maintain the same dietary patterns the evening before each test. The experimental exercise protocol (Figure 4.1) was adopted with slight modifications from a previously published paper by Thivel et al (Thivel, Metz, Julien, Morio, & Duche, 2014). Prior to the test, subjects were fitted
with a heart rate monitor (Polar, Kempele, Oulu, Finland). Participants were asked to complete the baseline questionnaire and then drink 250 mL of water (within 5 min) followed by the insertion of an IV catheter. For the HIEX session, at 39 min participants were seated on the recumbent bicycle and asked to start peddling (at 25 Watts and 60-70 RPM) for one minute before the first HIEX bout started. The HIEX protocol consisted of three 10-minute bouts of HIEX at 70% \( \text{VO}_2 \text{peak} \) at 60-70 RPM with 1:30 minute active rest interposed at 25 Watts with 60-70 RPM. For the resting session, participants spent the entire time resting, reading a book or doing homework. The first blood sample was drawn at baseline (0 min), at 50 min, at 80 and at the end of the study at 110 min. To ensure consistency, each child attended experimental sessions after a 12-hour fast, at the same time and day of the week, one week apart.

### 4.2.4. Visual Analog Scales

Visual Analog Scales were employed to assess subjective appetite based on the following questionnaires: “Determination to Eat” (DTE), “Hunger”, “Fullness” and “Prospective Food Consumption” (PFC). The calculation and methodology can be found in Appetite scores were calculated as a function using the following formula: Average Appetite score (mm) = [DTE + hunger + (100 - fullness) + PFC]/4 (G. H. Anderson, Catherine, Woodend, & Wolever, 2002). Physical Comfort, Nausea, and Thirst were also measured to determine possible confounders for the appetite scores as previously described (Bozinovski et al., 2009; Hunschede et al., 2015; Tamam et al., 2012). Furthermore, Nausea has been shown to be associated with exercise-induced anorexia (Kondo et al., 2001). Participants were instructed to read each question and place an “X” along a 100-mm line depending on how they felt at the current moment. Visual
analog scale questionnaires were administered at baseline (0 min), 10, 50, 65, 80, 95 and 110 min after the start of each session.

4.2.5. Blood collection and plasma sample processing

Blood samples were collected into pre-chilled 10 mL BD Vacutainer™ (BD Diagnostics, Sparks, MD, USA) blood collection tubes containing spray-dried K₂EDTA anticoagulant, and a proprietary cocktail of protease inhibitors [e.g. DPP-IV (R-3-Amino-1-{3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazol[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-on), AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride) and aprotinin (Trasylol)] to prevent the proteolytic breakdown of hormones. Immediately after collection, plasma was separated by centrifugation for 15 min at 2000 relative centrifugal force at 4 °C, then aliquotted into 2 mL Eppendorf (Eppendorf, Hamburg, Germany) tubes and stored at -80 °C for later analyses. In addition, to enhance the active ghrelin stability in blood samples, 200 µL 1 N HCl was added to every 1 ml of plasma collected for ghrelin analysis.

4.2.6. Biochemical plasma measurements

Plasma levels of glucose, insulin, CRP and cortisol were analyzed at Mount Sinai Hospital (Mount Sinai, Toronto, ON, Canada). All other analyses were performed in the Department of Nutritional Sciences at the University of Toronto. Leptin, total PYY [i.e. PYY (1-36 amide) and PYY (3-36)] and the biological active form of ghrelin and GLP-1 [i.e. GLP-1 (7-36 amide)] were analyzed by using commercial ELISA kits purchased from Millipore (Millipore, Billerica, MA, USA): (Leptin
cat. #EZHL-80SK, Sensitivity: 0.5 ng/mL, Range: 0.5–100 ng/mL; active ghrelin cat. #EZGRA-88K, Sensitivity: 8pg/mL, Range: 25-2000 pg/mL; GLP-1 cat. #EGLP-35K, Sensitivity: 2pM, Range: 2-100 pM; PYY cat. #EZHPYYT66K, Sensitivity: 6.5 pg/mL, Range: 14-1800 pg/mL). Interleukin-6 and TNF-α were analyzed using sandwich ELISA kits from R&D systems (R&D Systems, Minneapolis, MN, USA); (IL-6 cat. #HS600B, Sensitivity: 0.11 pg/mL, Range: 0.156 - 10 pg/mL; TNF-α cat. #HSTA00D, Sensitivity: 0.191 pg/mL, Range: 0.5 - 32 pg/mL). For all assays intra-CV was <4%, and Inter-CV was <8%.

### 4.2.7. Statistical Analysis

All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, N.C., USA). The data were tested for normality using the SAS PROC UNIVARIATE procedure. Student’s t-test was used to compare subject characteristics, baseline VAS and baseline biomarker measurements for NW vs. OW/OB subjects. Baseline measurements are presented as absolute mean ± SEM. Visual analog scale and blood biomarker changes over time were analyzed using a 3-factor ANOVA by SAS PROC MIXED procedure followed by Tukey–Kramer’s post hoc test, with ACTIVITY (HIEX vs. rest), WEIGHT STATUS (NW vs. OW/OB) and TIME, as independent variables. Visual Analog Scale and blood biomarkers over time are presented as mean ± SEM and %mean ± SEM change from baseline, to allow a direct comparison between NW and OW/OB participants. For clarity, combined data for NW and OW/OB subjects are presented in results if no main effect of WEIGHT STATUS or interaction with WEIGHT STATUS was detected. Pearson correlation analysis was conducted between mean percent changes from baseline of each biomarker (Leptin, Active Ghrelin, PYY, GLP-1, Blood Glucose, Insulin, CRP, IL-6, TNF-α, and Cortisol) and each of the
mean change in VAS scores (Appetite, DTE, Fullness, Hunger, and PFC). Statistical significance was declared at $P < 0.05$. 
4.3. Results

Twenty-two participants, 11 NW and 11 OW/OB boys completed the study. Overweight and OB participants had higher body weight \((p = 0.012)\), BMI \((p < 0.001)\), BMI percentile \((p < 0.001)\) and %body fat \((p < 0.001)\) but lower physical fitness \((\text{VO}_2\text{peak})\) \((p < 0.001)\) compared to NW. Participants’ age \((p = 0.464)\), height \((p = 0.862)\), Tanner stage \((p = 0.302)\), heart rate (HR) during the HIEX bout \((\text{HR}_{\text{EX}})\) \((p = 0.898)\), and the maximum HR during the screening session \((\text{HR}_{\text{MAX}})\) \((p = 0.99)\) were not significantly different \((\text{Table 4.1})\).

4.3.1. Heart Rate

Heart rate increased with HIEX \((p < 0.001)\), WEIGHT STATUS \((p < 0.001)\) and TIME \((p < 0.001)\). Heart rate during HIEX \((40-73 \text{ min})\) did not differ between NW and OB boys \((p = 0.898)\) \((\text{Figure 4.2})\).

4.3.2. Baseline Appetite, Physical Comfort Scores, and Blood Parameters

At baseline, appetite \((p = 0.026)\) and PFC \((p = 0.009)\) scores were lower, and fullness ratings were higher \((p < 0.001)\) in OW/OB boys when compared to NW boys \((\text{Table 4.2})\). Visual Analog Scales for Hunger, DTE, COM, Nausea, and Thirst were not significantly different.

Overweight and OB boys had higher levels of circulating baseline leptin \((p < 0.001)\), blood glucose \((p < 0.001)\), insulin \((p = 0.003)\) and HOMA-IR \((p = 0.005)\) compared to NW boys \((\text{Table}
No differences in baseline levels were found for active ghrelin, GLP-1, PYY, Cortisol, CRP, IL-6, and TNF-α.

4.3.3. Visual Analog Scale Appetite Response to High Intensity Exercise

HIEX decreased appetite \( (P = 0.04) \) and DTE \( (P = 0.019) \) compared with rest, with no effect by WEIGHT STATUS \( (P > 0.299) \) and increased over TIME \( (P < 0.001) \) (Figure 4.3A). There was an ACTIVITY×WEIGHT STATUS interaction for DTE \( (P = 0.043) \), resulting from OW/OB subjects being less hungry than NW after HIEX (Figure 4.3C). Prospective food consumption was affected by neither ACTIVITY \( (P = 0.346) \) nor by WEIGHT STATUS \( (P = 0.327) \) but was increased over TIME \( (P < 0.001) \) (Figure 4.3D). Fullness was not affected by either ACTIVITY \( (P = 0.29) \), WEIGHT STATUS \( (P = 0.85) \) or TIME \( (P = 0.072) \) (Figure 4.3E). Physical comfort was not affected by ACTIVITY \( (P = 0.081) \) or WEIGHT STATUS \( (P = 0.51) \) but decreased over TIME \( (P = 0.017) \). Thirst and Nausea were not affected by ACTIVITY, WS, or TIME. No other interactions were found.

4.3.4. Plasma Appetite Hormone Response to High-Intensity Exercise

Plasma leptin levels, shown as percent change from baseline, were not affected by ACTIVITY \( (P = 0.835) \), WEIGHT STATUS \( (P = 0.389) \) or TIME \( (P = 0.008) \). An interaction was found for ACTIVITY×WEIGHT STATUS \( (P < 0.001) \), showing that leptin response to HIEX was increased and more consistent compared to rest in NW than OW/OB children (Figure 4.4A). Plasma active ghrelin levels were decreased after HIEX \( (P < 0.001) \) independent of WEIGHT STATUS \( (P = 0.808) \) and affected by TIME \( (P = 0.013) \). There was an interaction of ACTIVITY×TIME \( (P = 0.03) \) with
plasma ghrelin levels decreasing in response to HIEX (0-50 min) and returning to baseline levels at 110 min (Figure 4.4B). Plasma PYY levels were not affected over TIME (P = 0.26), ACTIVITY (P = 0.391) or WEIGHT STATUS (P = 0.392). No other interactions were found (Figure 4.4C). Glucagon-like-peptide-1 was not affected by ACTIVITY (P = 0.527), TIME (P = 0.143), WEIGHT STATUS (P = 0.408) or their interactions (Figure 4.4D).

4.3.5. Plasma Glucose and Insulin Response to High-Intensity Exercise

Blood glucose levels (% change from baseline) were increased by HIEX (P = 0.002) and decreased over TIME (P < 0.001) with an interaction between ACTIVITY×WEIGHT STATUS (P = 0.032). The interaction is explained by an increase in glucose after HIEX and decrease at rest in NW children with little effect in OW/OB (Figure 4.5A). Insulin was also increased by HIEX (P = 0.028) and decreased by TIME (P < 0.001), with an interaction between ACTIVITY×WEIGHT STATUS (P = 0.001). Again, the interaction is explained by its increase after HIEX and decrease at rest in NW but not OW/OB children (Figure 4.5B).

4.3.6. Plasma Inflammatory and Stress Responses to High Intensity Exercise

Cortisol was increased with HIEX (P < 0.001) and over TIME (P = 0.018), but not affected by WEIGHT STATUS (P = 0.408). There was a TIME×ACTIVITY interaction (P < 0.001) due to a 142% increase from baseline during HIEX (30-80 min) followed by a decrease from 50-110 min to baseline levels (Figure 4.6A). C-reactive protein showed a trend to increase with HIEX (P = 0.072) but was not affected by WEIGHT STATUS (P = 0.703) or TIME (P = 0.707) (Figure 4.6B). Interleukin-
6 was increased by HIEX (P < 0.001) and over TIME (P = 0.026) but was not affected by WEIGHT STATUS (P = 0.713). There was an interaction of ACTIVITY×TIME (P = 0.014) due to a 120% increase of IL-6 from 30 to 110 min when compared with rest (Figure 4.7C). Tumor necrosis factor-α also increased with HIEX (P < 0.001) and over TIME (P = 0.003) but was not affected by WEIGHT STATUS (P = 0.097). (Figure 4.6D). No other interactions were found.

4.3.7. Associations between Appetite and Biomarkers of Appetite, Inflammation, and Stress

No significant correlations were found between each of mean % changes in appetite, hunger, fullness, DTE, PFC and each of mean % change of Leptin, PYY, GLP-1, Cortisol, CRP and TNF-α. However, IL-6 was inversely correlated with appetite (r = −0.379; P = 0.012) and Fullness (r =0.446; P = 0.004). Active ghrelin was inversely correlated with fullness (r =−0.341; P = 0.025) but not appetite. Blood glucose was positively correlated with fullness (r = 0.326; P = 0.035). Correlations were negative between Leptin and GLP-1 (r = -0.477; P = 0.001) and active ghrelin and cortisol (r = -0.352; P = 0.021) but positive between TNF-α and IL-6 (r = 0.337; P = 0.027).
4.4. Discussion

HIEX suppressed appetite in boys as previously shown in adults (J. E. Blundell & King, 2000; Sim et al., 2013). In addition, the hypothesis that an acute suppression of appetite after HIEX (at 70% VO₂ peak) is associated with IL-6, but not appetite hormone responses, was partially supported. First, IL-6 increased while appetite VAS scores were suppressed after HIEX and IL-6 was the only biomarker that correlated with appetite. Second, appetite hormones leptin, PYY and GLP-1 were not affected by HIEX and did not correlate with appetite. However, HIEX increased cortisol and decreased active ghrelin that contribute to food intake regulation, but neither correlated with appetite scores.

Because IL-6, but not TNF-α or CRP, matched the time-course of VAS appetite scores and correlated with VAS scores for appetite and fullness, a possible role for IL-6 in the relationship between appetite post-HIEX can be suggested. IL-6 and its role in HIEX have been investigated since 1991 (Northoff & Berg, 1991). Although both TNF-α and IL-6 are high during sepsis and cachexia and derived predominately by macrophages, exercise induced an increase in IL-6 produced by the muscle (Pedersen & Febbraio, 2008). Although TNF-α was increased with HIEX from rest, the difference arose primarily from a decrease in TNF-α during rest, perhaps mediated by diurnal changes (Muc-Wierzgon, Madej, Baranowski, & Wierzgon, 1998). CRP was measured because it is also a sensitive marker of inflammation, often used to quantify low systemic inflammation in normal-weight and obese individuals (Kapiotis et al., 2006) and has been positively associated with the loss of appetite and weight loss during cancer (Kalantar-Zadeh et al., 2004). However, it was not different between the BW groups and its lack of change with HIEX is consistent with the fact that it is known to be a tonic indicator of systemic inflammation and is
less variable to acute stimuli such as exercise (De Jongh et al., 2003). CRP was also not correlated with appetite.

The results of this series of experiments support a previous review which concluded that appetite hormones have only negligible or small effects in suppression of appetite after HIEX (Schubert et al., 2014). Leptin, PYY, and GLP-1 were not affected by HIEX (70% VO\textsubscript{2}peak), similar to studies in adults (Almada et al., 2013; Croft et al., 2009; Flegal et al., 2002; Pescatello & American College of Sports Medicine., 2014; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Wernstedt et al., 2006) and did not correlate with appetite. Furthermore, leptin, PYY, and GLP-1 did not match the time course of appetite nor correlate with any of the VAS measurements. This is not surprising because most studies reporting an effect of appetite hormones on FI are based on the effects of peripheral injections resulting in alterations of appetite and EI (Batterham et al., 2003; Druce et al., 2006; Friedman & Halaas, 1998; Turton et al., 1996).

HIEX induced changes in ghrelin and cortisol occurred, but their contribution to the decrease in appetite is unclear. HIEX decreased active ghrelin while it steadily increased during rest, which has been confirmed by previous studies (Wasse et al., 2013), but was not correlated with overall average appetite. However, the decrease in active ghrelin correlated with an increase in one component of the appetite scale, fullness, making it a possible candidate for the HIEX induced suppression of appetite. It is unknown, how the effect of HIEX on active ghrelin secretion associates with fullness. However, HIEX increases sympathetic nervous activity and facilitates the redistribution of splanchnic blood flow to the peripheries (Otte, Oostveen, Geelkerken, Groeneveld, & Kolkman, 2001), leading to gastric mucosal ischemia, possibly resulting in a decreased secretion and lower ghrelin plasma levels (Perini & Veicsteinas, 2003).
This process of blood-flow re-distribution is necessary to meet the increased energy demand imposed by HIEX. Ghrelin has also previously been shown to be inversely associated with cortisol (Espelund et al., 2005). In our study, blood cortisol mirrored active ghrelin levels, increasing to 150% at 50 min with HIEX, and declined from 50 to 110 min, similar to another study in adults (Duclos, Gourne, & Bonnemaison, 2003). Plasma cortisol levels were not correlated with appetite VAS or any other appetite markers. The injection of cortisol has been shown to decrease food intake, and the anorexic effect of cortisol has been hypothesized to act via two possible pathways, 1) The decrease of ghrelin receptor expression in the hypothalamus and ghrelin secretion and 2) a decreased NPY expression (Janzen et al., 2012). However, exact mechanisms of how cortisol can affect appetite need to be elucidated.

Blood glucose and insulin are well-described biomarkers of appetite (Lemmens, Martens, Kester, & Westerterp-Plantenga, 2011). During exercise blood, glucose levels are usually stable in healthy individuals (Hunschede et al., 2015). However, obese display higher insulin, glucose and HOMA-IR levels at baseline (Table 2), indicating increased insulin resistance and metabolic inflexibility (Chiarelli & Marcovecchio, 2008). This may lead to increased blood glucose and insulin levels in OW/OB when compared to NW during and after high-intensity exercise, resulting in increased hyperglycemia in obese when compared to NW participants. However, while blood glucose differed between NW and OW/OB, appetite responses did not, suggesting its response to HIEX was not driving the appetite response.

This study had several limitations that future research must address. First, appetite was measured but not EI. Currently, there is only limited data available on the suppression of appetite in children. The time course of appetite and hormonal response to HIEX is largely unknown in
NW and OW/OB children. EI was not measured in this study to investigate the time point of the strongest appetite suppression within this 1-hr time period. Furthermore, previous research has shown an uncoupling effect of EI and VAS appetite scores in NW and OW/OB children and adolescents (Thivel & Chaput, 2014) and therefore future studies need to assess EI. Second, while the results show, that IL-6 is correlated with appetite after HIEX in boys, this association does not indicate a functional relationship. Studies with varying levels of IL-6 or IL-6 antagonists are needed to further understand the role if IL-6 in appetite control. Finally, the effect of gender on the exercise-induced suppression of appetite was not addressed. Adult females have an attenuated inflammatory response to exercise (Stupka et al., 2000), and may increase EI in response to HIEX (Pomerleau, Imbeault, Parker, & Doucet, 2004).
4.5. Conclusion

HIEX resulted in reduced appetite that correlated with an increase in IL-6 in both NW and OW/OB boys. However, while a role for IL-6 in the response can be suggested, the suppression of appetite was potentially mediated by the decrease in active ghrelin and/or increase in cortisol.
4.6. Acknowledgments

We would like to express our gratitude to the parents and children enrolled in the study for their participation; and the volunteers who assisted in the carrying out the study. This study was supported by the Canadian Institute for Health Research (grant/funding no. 490408). Clinical Trial Registry: Clinicaltrials.gov ID: NCT02619461. Authors’ contributions to the manuscript: Sascha Hunschede designed and conducted the experiment, analyzed the data and wrote the research paper. Dr. Ruslan Kubant and Dr. Rajadurai Akilen contributed to the analysis of the data and editing process of the manuscript. G. Harvey Anderson and Scott G. Thomas conceptualized, designed, and supervised the experiment, and had primary responsibility for the final content.
4.7. Tables and Figures

**Table 4.1: Participant Characteristics**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>NW ± SEM</th>
<th>OW/OB ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>15.4 ± 0.8</td>
<td>14.5 ± 0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.6 ± 4.8</td>
<td>80.9 ± 5.5*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.5 ± 3.8</td>
<td>165.6 ± 3.4</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>21.5 ± 1.2</td>
<td>29.2 ± 1.3***</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>59.8 ± 8.2</td>
<td>96.4 ± 0.7***</td>
</tr>
<tr>
<td>VO₂peak (mL·kg⁻¹·min⁻¹)</td>
<td>43.5 ± 2.3</td>
<td>30.9 ± 1.7***</td>
</tr>
<tr>
<td>%VₖT of VO₂peak</td>
<td>63.2 ± 3.3</td>
<td>57.8 ± 2.7</td>
</tr>
<tr>
<td>HRₘ₅X (BPM)</td>
<td>187 ± 3.0</td>
<td>187 ± 2.4</td>
</tr>
<tr>
<td>HRₑxing (BPM)</td>
<td>151 ± 5.3</td>
<td>152 ± 5.6</td>
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<tr>
<td>Body fat (%)</td>
<td>20.1 ± 1.7</td>
<td>39.3 ± 1.1***</td>
</tr>
<tr>
<td>Tanner Stage</td>
<td>3.5 ± 0.4</td>
<td>2.9 ± 0.4</td>
</tr>
</tbody>
</table>

Note: BMI, body mass index; VO₂peak, maximum oxygen consumption; %VₖT, ventilation threshold percentage of VO₂peak; HRₘ₅X, maximum average heart rate achieved at the screening; HRₑxing, average heart rate achieved during HIEX sessions from 40-73 min. Values are means ± SEM; n = 22 (n = 11 NW and n = 11 OW/OB). Significantly different from NW by unpaired t test (*P < 0.05; **P < 0.01; ***P < 0.001).
Table 4. 2: Baseline Measurements in NW and OW/OB

<table>
<thead>
<tr>
<th>Measurement</th>
<th>NW ± SEM</th>
<th>OW/OB ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual Analog Scales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appetite (mm)</td>
<td>68.8 ± 4.7</td>
<td>53.3 ± 2.7*</td>
</tr>
<tr>
<td>DTE (mm)</td>
<td>55.6 ± 6.7</td>
<td>48.7 ± 4.3</td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>56.0 ± 6.0</td>
<td>49.5 ± 3.7</td>
</tr>
<tr>
<td>PFC (mm)</td>
<td>71.6 ± 5.9</td>
<td>53.3 ± 3.3**</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>19.9 ± 3.8</td>
<td>38.1 ± 3.1*</td>
</tr>
<tr>
<td><strong>Plasma Biomarkers of Appetite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>3.8 ± 0.7</td>
<td>11.3 ± 1.5***</td>
</tr>
<tr>
<td>Ghrelin active (pg/mL)</td>
<td>7.3 ± 1.3</td>
<td>8.3 ± 1.1</td>
</tr>
<tr>
<td>PYY (pg/mL)</td>
<td>3.7 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>GLP-1 (pM)</td>
<td>5.4 ± 0.9</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.1**</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>71.3 ± 7.2</td>
<td>124.2 ± 17.6**</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3 ± 0.2</td>
<td>4.4 ± 0.7**</td>
</tr>
<tr>
<td><strong>Plasma Biomarkers of Stress and Inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>326.4 ± 29.2</td>
<td>296.3 ± 44.8</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.9 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.3 ± 0.4</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

Note: NW, Normal-weight; OW, Overweight; OB, Obese; DTE, Determination to eat; PFC, Prospective food consumption; PYY, Peptide Tyrosine Tyrosine; GLP-1, Glucagon-like-peptide-1; HOMA-IR, Homeostatic model assessment for insulin resistance; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor alpha. Values are means ± SEM; n = 22 (n = 11 NW and n = 11 OW/OB). Significantly different from NW by unpaired t-test (*P < 0.05; **P < 0.01; ***P < 0.001).
Figure 4.1: Study protocol flow diagram. Note: VAS = Visual analog scale; light grey area = 10 min HIEX bout; dark grey area = 1:30 min rest; blood drop = time point for each blood draw.
Figure 4.2: Heart rate during HIEX and rest. Values are Mean ± SEM, n=22 (11 NW and 11 OW/OB).
Figure 4.3: Change from baseline in Appetite (A), Determination to Eat (B), Hunger (C), Prospective Food Consumption (D) and Fullness (E) VAS response to HIEX at 0, 50, 65, 80, 95 and 110 min. Values are Mean ± SEM, n=22 (11 NW and 11 OW/OB). Combined data for NW and OW/OB subjects are presented in results if no main effect of WEIGHT STATUS or interaction with WEIGHT STATUS was detected.
Figure 4.4: Change from baseline plasma levels of Leptin (A), Active Ghrelin (B) and Peptide YY (C) in response to HIEX at 0, 50, 80, 110 min. Asterix (*) denotes values that are significantly different (HIEX vs. rest) at each time point (3-way ANOVA, Tukey–Kramer post hoc test, p < 0.05). Values are Mean ± SEM, n=22 (11 NW and 11 OW/OB). Note: For clarity, combined data for NW and OW/OB subjects are presented in results if no main effect of WEIGHT STATUS or interaction with WEIGHT STATUS was detected.
Figure 4.5: Change from baseline plasma levels of Blood Glucose (A) and Insulin (B) in response to HIEX at 0, 50, 80- and 110-min. Statistical significance (HIEX vs. rest) is denoted by asterix (*) (3-way ANOVA, Tukey–Kramer post hoc test, p < 0.05). Values are Mean ± SEM, n=22 (11 NW and 11 OW/OB).
Figure 4. 6: Change from baseline plasma levels of IL-6 (A) and TNF-α (B) in response to HIEX at 0, 50, 80 and 110 min. Dagger (†) denotes values that are significantly different for NW (HIEX vs. rest) at each time point (3-way ANOVA, Tukey–Kramer post hoc test, $p < 0.05$). Values are Mean ± SEM, n=22 (11 NW and 11 OW/Ob). Note: For clarity, combined data for NW and OW/Ob subjects are presented in results if no main effect of WEIGHT STATUS or interaction with WEIGHT STATUS was detected.
Chapter 5
THE ROLE OF IL-6 IN EXERCISE-INDUCED ANOREXIA IN NORMAL-WEIGHT BOYS.

The following chapter is a reproduction of a manuscript that has been published in Applied Physiology, Nutrition and Metabolism Vol. 43, Issue 10; March 2018.
Title: The role of IL-6 in exercise-induced anorexia in normal-weight boys

Running Title: IL-6 and Appetite Regulation in Children.

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Abstract:

Background: Our previous study showed that Interleukin-6 (IL-6) is associated with a suppression of appetite after high-intensity exercise (HIEX), but an independent role within food intake (FI) was not defined.

Hypothesis: IL-6 after HIEX (75% VO2peak) is independent of appetite-hormones in suppressing appetite and FI in normal-weight (NW) boys.

Objective: To investigate the effect of HIEX, with and without the inflammation inhibitor, ibuprofen (IBU), on IL-6 and selective biomarkers of inflammation and appetite on FI and ratings of appetite in NW boys.

Design: 15 NW boys (aged 13-18y) were randomly assigned in a crossover design to four sessions: 1)Water+Rest; 2)Rest+IBU; 3)Water+HIEX; 4)HIEX+IBU. HIEX consisted of three 10min bouts of HIEX at 75% VO2max with 1:30min active rest interposed. IBU was given in a 300mg liquid solution. EI, ratings and plasma biomarkers of appetite, inflammation, stress and glucose control were measured.

Results: FI was not affected by HIEX or IBU. Appetite increased over TIME (p=0.002) but was lower after HIEX (p<0.001) with no effect of IBU. HIEX, but not IBU, also resulted in higher levels of IL-6 (p<0.001) and cortisol (P<0.001), and lower active ghrelin (P<0.001). IL-6 correlated with active ghrelin (r=0.37; p=0.036) and Cortisol (r=0.26; p=0.049).

Conclusion: HIEX reduces subjective appetite but not EI, accompanied by an increase of IL-6 and cortisol and a decrease of active ghrelin and blood glucose. An independent role for IL-6 in appetite suppression was not supported. However, IL-6 was associated with active ghrelin and cortisol, thus potentially mediating appetite via these interactions.
5.1. Introduction:

Information regarding energy status, hunger and satiety are communicated between the brain and the gut via appetite hormones. These episodic signals arise in response to macronutrients in the intestinal system and are thought to be involved in appetite regulation during and after exercise. Although high levels of prolonged activity should initiate hunger, exercise at high intensities suppresses appetite and food intake (FI) (J. E. Blundell, Gibbons, Caudwell, Finlayson, & Hopkins, 2015; J. E. Blundell & King, 2000; N. A. King et al., 1994; Sim et al., 2014).

This high-intensity exercise (HIEX) induced anorexia is dependent on exercise modality (Panissa et al., 2016), duration (Bozinovski et al., 2009), and the time between the cessation of exercise and initiation of FI (N. A. King et al., 1994). However, the mechanisms are not well understood. Appetite hormones have been reported to be responsible for the post-exercise reduction in appetite in some (G. H. Anderson et al., 2016; Hazell, Islam, Townsend, Schmale, & Copeland, 2016) but not other studies (Larson-Meyer et al., 2012; Unick et al., 2010). Biomarkers from the stress and inflammatory system also respond to HIEX. Interleukin-6 (IL-6) has been associated with suppression of appetite after HIEX in adults (Almada et al., 2013) and boys (Hunschede, Kubant, A., Thomas, & Anderson, 2017). In our previous study, IL-6 correlated with reduced subjective appetite and fullness VAS, but active ghrelin also correlated with fullness VAS (Hunschede, Kubant, A., et al., 2017) making an independent role for IL-6 uncertain.

IL-6 is an inflammatory cytokine and its increase in plasma is believed to be related to muscle micro-damage (Bruunsgaard et al., 1997). It is secreted by the muscle, confirming its role as a myokine (Pedersen, Ostrowski, Rohde, & Bruunsgaard, 1998; Pedersen et al., 2004; Petersen...
The increase of systemic IL-6 after HIEX has been a consistent finding, and its appearance in plasma precedes other inflammatory cytokines (Pedersen et al., 1998). The peak of IL-6 induced by HIEX is reached shortly after the end of exercise (Pedersen, Steensberg, & Schjerling, 2001) and has been associated with decreased appetite (Almada et al., 2013; Hunschede, Kubant, A., et al., 2017). Several animal studies have also shown decreased appetite after high levels of IL-6 were induced by lipopolysaccharide (LPS) (Boelen et al., 1995) or direct IL-6 (K. Wallenius et al., 2002) and TNF-α injections (Langhans & Hrupka, 1999). Conversely, IL-6 deficient mice display obesity as early as ten weeks of age, due to increased FI but not EE (Chida et al., 2006).

Further evidence for a potential role of cytokines in appetite control is provided by their known transport across the blood-brain barrier, where they interact with the luminal surface of brain endothelial cells to release substances that affect appetite (Banks, 2001). They inhibit appetite directly by stimulating hypothalamic neurons sensitive to glucose in the lateral hypothalamic area (Plata-Salaman, 1996) by suppression of neuropeptide systems such as corticotrophin-releasing factor or neuropeptide-Y (Laviano et al., 1996; Plata-Salaman, 1991).

Ibuprofen or isobutylphenylpropanoic acid (IBU) is a nonsteroidal anti-inflammatory drug (NSAID) used for treating pain, fever by reducing the acute inflammatory response. Athletes and recreational exercisers often take IBU to decrease muscle soreness, cell injury and the inflammatory response after exercise (Mahler, 2001). An oral dose of IBU decreases circulating levels of cytokines such as IL-6, TNF-α and CRP within the 30-45 minutes after ingestion (Barnes et al., 2014; Gallelli et al., 2013). However, its effect on appetite and FI has not been reported.
Therefore, the objective of this study was to determine the effect HIEX, with and without IBU consumption, on IL-6, Fl regulatory hormones, appetite, and Fl in normal-weight (NW) boys.
5.2. Materials and Methods:

5.2.1. Participants

Fifteen NW (BMI for age percentile: 15th-85th) boys aged 13-18yrs completed the study. Sample size was estimated using appetite VAS scores from our previous studies, assuming that differences in VAS scores will translate to differences in FI. A telephone questionnaire was employed to determine eligibility for this study. Boys who answered “yes” to one of the questions of the physical activity readiness questionnaire, displayed a fear of venipuncture, dieters, had been diagnosed with diabetes or other metabolic diseases, and those scoring ≥11 on an Eating Habit Questionnaire were excluded from the study. All experimental procedures were approved by the University of Toronto Health Sciences Research Ethics Board, and informed consent was obtained from all adult participants, parents of the children as well as assent from the children themselves. Originally 21 participants were recruited through local advertisement at the University of Toronto and Toronto subway stations. However, six participants did not complete the study, due to either a mild form of vasovagal syncope likely due to the IV-catheter insertion, or difficulties scheduling the sessions.

Figure 1.

5.2.2. Participant Assessment

Participants were asked to come to the University of Toronto - Goldring Centre for High-Performance Sport for the initial screening. Age, height, body-weight, BMI, BMI for age percentile and percent body-fat were determined. Physical fitness was assessed by indirect calorimetry during continuous incremental cycling protocol on a Kettler RE7 recumbent bicycle (Kettler, Ense-
Parsit, NRW, Germany). Participants cycled for 3 min at 25 Watts, then the intensity was increased every minute by 20 watts. Ventilatory gases were collected using a Moxus metabolic cart (AEI Technologies Inc., Pittsburgh, PA, USA), a facemask, and a 2-way non-rebreathing valve (Hans Rudolph, Inc., Shawnee, KS, USA). Inspiratory ventilation was measured with a pneumotachometer, the O₂ and CO₂ contents of mixed expired gas with an S-3A Oxygen Analyzer, and CO₂ content with a CD-3A 251 Carbon Dioxide Analyzer (AEI Technologies Inc., Pittsburgh, PA, USA). Known gas concentrations of 16.04% O₂ and 4.06% CO₂ and 20% O₂ and 0.03% CO₂ were used to calibrate the metabolic cart, prior to each test. The Moxus metabolic cart has been validated over a wide measurement range using two sensors for ventilation against the Douglas bag method (Rosdahl, Lindberg, Edin, & Nilsson, 2013). VO₂peak was determined using the highest six consecutive breaths (Hunschede et al., 2015). Participants were included in the study if they exhibited normal body-weight (15th-85th BMI for age percentile) according to the Center for Disease Control and Prevention (2000) growth charts (Flegal et al., 2002). Bioelectrical impedance analysis was used to estimate body fat mass and fat-free mass (RJL Systems, Inc., Clinton Township, MI, USA) based on the Horlick equation (Horlick et al., 2002). All anthropometric measurements during the assessment are displayed in Table 5.1.

5.2.3. Study design and Experimental Protocol

Sample size for subjective appetite response to HIEX was estimated at N=14, using a within-subject design with α = 0.05(Z = – 0.025 = 1.96); β = 0.20 (Z = 0.80 = 0.84); σ = 20.7 mm and Δ = 8.7 mm in appetite VAS observed between control and treatment based on a previous
study (Hunschede, Kubant, Akilen, Thomas, & Anderson, 2016). \( \sigma \) represents standard deviation, \( \Delta \) represents the minimal difference, \( N \) is the number of subjects needed.

\[
N = 2 \times \left( \frac{(Z\alpha - Z\beta) \times \sigma}{\Delta} \right)^2
\]

All participants completed four sessions in a randomized order: (i) rest and water (ii) or exercise water, 30 min of HIEX at 75% VO\(_2\)peak or (iii) rest and IBU, 300 mg of Motrin IBU solution for children (Johnson and Johnson, New Brunswick, NJ, United States) or (iv) exercise and IBU, 30 min of HIEX at 75% VO\(_2\)peak and 300 mg of Motrin IBU solution. Both water control and IBU solution contained 0.8 g of sucralose (Heartland Food Products Group, Amsterdam, HP, Netherlands) and 1.2 g of orange flavoured Kool-Aid (Kraft Foods, Northfield, IL, United States) to mask the taste of the Motrin solution. To match sweetness and calorie content of the IBU Motrin drink, the water control also contained 6 mL high fructose corn syrup (ACH Food Companies, Mississauga, ON, Canada). To achieve peak IBU plasma levels during the HIEX session, the IBU treatment was given 20 min before the initiation of the HIEX session. Previous research has shown that IBU reaches its peak plasma levels in circulation after 30-40 min (Scott et al., 1999), thus being fully absorbed in the bloodstream halfway through the HIEX session.

Sessions were conducted on weekends between 9 and 10 am after a 12-h overnight fast. Parents were asked to encourage their children to drink water up to 1-h before the scheduled session, to refrain from physical activity, and to maintain the same dietary patterns the evening before each test.

**Figure 5.1**
5.2.4. Visual Analog Scales and Food Intake

Visual Analog Scales (VAS) were employed to assess subjective appetite based on the 100 mm lines anchored by extreme statements: “Determination to Eat” (DTE), “Hunger”, “Fullness” and “Prospective Food Consumption” (PFC). Physical Comfort, Nausea and Thirst were also measured with VAS, again anchored with extreme statements to determine possible confounders for the appetite scores as previously described (Bozinovski et al., 2009; Hunschede et al., 2015; Tamam et al., 2012). Furthermore, Nausea has been shown to be associated with exercise-induced anorexia (Kondo et al., 2001). Participants were instructed to read each question and place an “X” along a 100-mm line depending on how they felt (e.g, not hungry to as hungry as I have ever been) at the current moment. Visual analogue scale questionnaires were administered at baseline (0 min), 10, 30, 65, 80, 95 and 120 min after the start of each session.

Participants were provided with ad libitum lunch meal, consisting of rice (Uncle Ben’s, Bolton, ON, Canada), beef meatballs (President’s Choice, Brampton, ON, Canada) and tomato sauce (Ragu, Mount Prospect, IL, USA) at 100 – 120 min. The meatballs were cut into small and uniform pieces and were mixed homogeneously with the other ingredients in a bowl. Each bowl contained a 479.5 g portion which provided 827.5 kcal, 30.8 g fat, 104.8 g carbohydrate and 30.2 g protein based on the compositional information provided by the manufacturers. Two portions were served in 10 min intervals and FI from the meal was calculated based on the weight consumed during the lunch. Participants were instructed to eat until comfortably full and stay seated for the duration of the meal. A 500ml bottle of spring water (Danone Crystal Springs, Quebec City, QC, Canada) was provided during the HIEX session and with the meal, and additional
bottles were supplied if requested. Water consumption was measured by the weight (g) consumed. All baseline VAS measurements are displayed in Table 1.

5.2.5. Blood Collection

Blood samples were collected into pre-chilled 10 mL BD Vacutainer™ (BD Diagnostics, Sparks, MD, USA) at baseline (0 min), 65 min and 95 min. Blood collection tubes contained spray dried K₂EDTA anticoagulant, and a proprietary cocktail of protease inhibitors [e.g. DPP-IV (R-3-Amino-1-{3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazol[4,3-a]pyrazin-7-yl}-4-(2,4,5-trifluorophenyl)butan-1-on), AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride) and aprotinin (Trasylol)] to prevent the proteolytic breakdown of hormones. Immediately after collection, plasma was separated by centrifugation for 15 min at 2000 RCF at 4 °C, then aliquoted into 2 mL Eppendorf (Eppendorf, Hamburg, Germany) tubes and stored at -80 °C for later analyses. In addition, to enhance the active ghrelin stability in blood samples, 200 µL 1 N HCl was added to every 1 ml of plasma collected for ghrelin analysis. All baseline blood measurements are displayed in Table 1.

5.2.6. Biochemical plasma measurements

Plasma levels of glucose, insulin, CRP and cortisol were analyzed by the clinical laboratory at Mount Sinai Hospital (Mount Sinai, Toronto, ON, Canada). All other analyses were performed in the Department of Nutritional Sciences at the University of Toronto. Leptin, total PYY [i.e. PYY (1-36 amide) and PYY (3-36)] and the biological active form of ghrelin and GLP-1 [i.e. GLP-1 (7-36
amide) were analyzed using commercial ELISA kits (Millipore, Billerica, MA, USA): (Leptin cat. #EZHL-80SK, Sensitivity: 0.5 ng/mL, Range: 0.5–100 ng/mL; active ghrelin cat. #EZGRA-88K, Sensitivity: 8pg/mL, Range: 25-2000 pg/mL; GLP-1 cat. #EGLP-35K, Sensitivity: 2pM, Range: 2-100 pM; PYY cat. #EZHPYYT66K, Sensitivity: 6.5 pg/mL, Range: 14-1800 pg/mL). Interleukin-6 and TNF-α were analyzed using sandwich ELISA kits from R&D systems (R&D Systems, Minneapolis, MN, USA); (IL-6 cat. #HS600B, Sensitivity: 0.11 pg/mL, Range: 0.156 - 10 pg/mL; TNF-α cat. #HSTA00D, Sensitivity: 0.191 pg/mL, Range: 0.5 - 32 pg/mL). For all assays, intra-CV was <4%, and Inter-CV was <8%.

5.2.7. Statistical Analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, N.C., USA). The data was tested for normality using the SAS PROC UNIVARIATE procedure. The randomization schedule to treatments was generated using the SAS PROC PLAN procedure (SAS Institute v. 9.4, Cary, NC, USA). Baseline characteristics are presented as absolute mean ± SEM. Visual analog scale and blood biomarker changes over time from baseline were analyzed using a 3-factor ANCOVA by SAS PROC MIXED procedure followed by Tukey–Kramer’s post hoc test, with ACT (HIEX vs. rest), DRK (IBU vs. Water) and TIME as independent variables, and baseline (0 min) as covariate variable. VAS and blood biomarkers over time are presented as analyzed, as mean ± SEM change from baseline until 95 min. Pre- and Post (95 – 120 min) lunch VAS scores were presented separately. Pearson correlation analysis was conducted between mean raw data of each biomarker, each of the mean raw data in VAS scores and FI. For clarity, the data is presented for each treatment arm. Statistical significance was declared at $P < 0.05$. 
5.3. Results

5.3.1. Food Intake and Water Consumption:

FI (kcal/kg) was not affected by HIEX or IBU. Water consumption (mL) was increased by HIEX (p < 0.001), but not affected by IBU. No other main effects or interactions were found.

Figure 5.2

5.3.2. Post-exercise Appetite Scores

No differences in pre- and post meal VAS scores among treatments were found for appetite, PFC, DTE, hunger or fullness.

Figure 5.3

5.3.3. Appetite Visual Analog Scales:

HIEX lowered appetite (p = 0.04), DTE (p = 0.024) and PFC (p = 0.021). IBU had no effect on VAS scores. Over TIME, APP (p = 0.002), DTE (p < 0.001) and hunger (p < 0.001) increased but neither fullness nor PFC were changed. TIME and HIEX interactions were found due to increase in appetite (p = 0.026), DTE (p = 0.009), hunger (p = 0.025) and PFC (p = 0.05) over time. No other main effects or interactions were found.

Figure 5.4
5.3.4. Thirst, Physical Comfort, Nausea and Palatability Visual Analog Scales:

Thirst was increased by HIEX ($p = 0.016$) and decreased by IBU ($p = 0.03$) and TIME ($p < 0.001$). Neither palatability ratings between DRK nor of the *ad libitum* lunch meal were affected by HIEX or IBU. Nausea and physical comfort rating were also not affected by HIEX or IBU. No other main effects or interactions were found.

5.3.5. Appetite Biomarkers:

HIEX decreased Active Ghrelin ($p < 0.001$), but did not affect GLP-1, PYY, Insulin or Blood Glucose. IBU increased GLP-1 ($p = 0.01$) but had no effect on Active Ghrelin, PYY, Blood Glucose or Insulin. Over TIME, Active Ghrelin ($p < 0.001$), Blood Glucose ($p < 0.001$) and Insulin ($p < 0.001$) steadily decreased while GLP-1 and PYY were unchanged. Active Ghrelin was lowest at 65 min and then increased to baseline levels at 95 min, explaining the TIME by HIEX interaction ($p < 0.001$). The ACT*DRK interaction ($p = 0.007$) and TIME*ACT interaction ($p = 0.014$) were due to the increased active ghrelin after HIEX and IBU compared with rest or water, respectively.

Figure 5.5

5.3.6. Inflammatory Biomarkers:

HIEX increased IL-6 ($p < 0.001$) and Cortisol ($p < 0.001$), but did not affect TNF-α or CRP compared with the rest condition. IBU had no effect on IL-6, TNF-α, Cortisol or CRP compared with the water control. Over TIME, IL-6 ($p < 0.001$) and Cortisol ($p = 0.021$) increased, but CRP
and TNF-α were not affected. TIME*ACT interactions were found for IL-6 (p = 0.001) showing an increase to 95 min and Cortisol (p = 0.002) to 65 min with HIEX when compared to rest. ACT*DRK interactions were found for CRP (p = 0.04) and TNF-α (p = 0.001). CRP was lower at 65 min, but rebounded to 95 min in the rest condition, whereas it was highest at 65 min and decreased to 95 min, after HIEX. IBU dampened the effect of HIEX at 65 min. Similarly, at 65 min the effect of DRK on TNF-α reversed the effect of the two ACT treatments.

Figure 5.6

5.3.7. Correlations

Ghrelin was correlated with Cortisol (r = -0.31; p = 0.018) and Insulin (r = 0.39; p = 0.002). GLP-1 was correlated with PYY (r = 0.47; p = 0.002). Insulin was correlated with Glucose (r = 0.39; p = 0.022), Appetite (r = 0.43; p < 0.001) and DTE (r = -0.43; p < 0.001). IL-6 was correlated with Ghrelin (r = 0.37; p = 0.036), TNF-α (r = -0.26; p = 0.045) and Cortisol (r = 0.26; p = 0.049).
5.4. Discussion:

IBU did not affect FI or appetite at rest or with exercise. The IL-6 response to HIEX was not significantly affected by IBU administration. However, this study suggests IL-6 may affect appetite in a correlated response with active ghrelin and cortisol. The study confirms previous reports showing that HIEX leads to suppression in appetite (J. E. Blundell & King, 2000; Sim et al., 2013), but in addition, we show that FI was not affected. Furthermore, IBU did not affect appetite or FI, although it demonstrated a significant improvement in inflammatory markers, appetite scores and body-weight, in patients with GI cancer (McMillan, O’Gorman, Fearon, & McArdle, 1997; McMillan et al., 1999).

Many studies have shown that HIEX increases IL-6 which is produced as a myokine by the muscle (Pedersen & Fischer, 2007) but has no effect on TNF-α which is produced by macrophages (Croft et al., 2009). However, the lack of effect of IBU on IL-6 was surprising. IBU is known to reduce IL-6 by inhibiting the cyclooxygenase enzymes (COX)-1 and COX-2 (Davies, 1998), thus preventing the formation of various prostaglandins (PGE), including PGE-2, that increase IL-6 production in macrophages in response to acute inflammation. However, we found IBU reversed the effect of ACT on CRP and TNF-α, thus confirming that the dose of 300mg IBU was sufficient to alter some inflammatory responses, but those responses did not correlate with appetite.

Although the present study does not show an independent role for inflammatory biomarkers contributing to a suppression of appetite, several potential mechanisms support continued investigation of cytokines role in appetite and FI regulation. During states of an increased inflammatory response, cytokines are transported across the blood-brain barrier and exert their effects on the luminal surface of endothelial brain cells causing secretion of
substances that affect appetite (Banks et al., 2011). TNF-α and IL-1 receptors in the hypothalamic area of the brain which regulate FI (Hellerstein et al., 1989) and intraperitoneal injections of human TNF-α receptor antagonist increase FI in tumour-bearing rats (Torelli et al., 1999) indicating a potential role in FI and appetite regulation.

In the present study, IBU reduced the TNF-α response to exercise but did not affect appetite, suggesting TNF-α did not play a role but perhaps because responses in these inflammatory biomarkers were many times lower than the ones observed in the tumour bearing rats. Finally, it has also been shown in rats that HIEX increases the permeability of the blood-brain barrier (BBB) (Sharma, Cervos-Navarro, & Dey, 1991), potentially increasing transport of appetite-regulating components that usually do not affect appetite and FI.

Appetite-regulating hormones are proposed to play a role in the control of appetite during and after exercise (G. H. Anderson et al., 2016), but not one hormone has been shown to be a consistent factor in the response. For example, several studies show that active ghrelin is decreased (Becker et al., 2012; Broom et al., 2007; J. A. King et al., 2010; J. A. King et al., 2011; Wasse et al., 2012; Wasse et al., 2013), while GLP-1 (Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009) and PYY (Broom et al., 2009; Wasse et al., 2012) are often increased after HIEX. However, others reported no effect of HIEX on GLP-1 (Larson-Meyer et al., 2012) and PYY (Kelly et al., 2012; Larson-Meyer et al., 2012) or even decreased levels of GLP-1 (Unick et al., 2010).

This inconsistent association of appetite hormones from the gut with HIEX induced anorexia may also be explained by decreased splanchnic blood flow after HIEX compared with an increase in intestinal blood flow after FI. During and after eating, splanchnic blood flow is
increased to aid digestion, releasing appetite-regulating hormones to signal nutrient availability (Austin & Marks, 2009). Blood flow in the celiac artery peaks rapidly at a 38–60% increase from fasting levels (Qamar, Read, Skidmore, Evans, & Williamson, 1985; Someya, Endo, Fukuba, & Hayashi, 2008), while the blood flow in the superior mesenteric artery increases 1.5- to 3.5-fold, 5–60 min after a meal (Qamar & Read, 1988; Sidery, Macdonald, Cowley, & Fullwood, 1991; Someya et al., 2008). In contrast, during HIEX splanchnic blood flow is decreased and redistributed from the gut to contracting skeletal muscle to increase oxygen supply (Eriksen & Waaler, 1994). HIEX at 70% VO2max decreased blood flow in the portal vein by 80% after 60 min of cycling (Rehrer, Smets, Reynaert, Goes, & De Meirleir, 2001), and by 43% after in the superior mesenteric artery after 30 min of treadmill running (Qamar & Read, 1987). This contrast in blood flow after HIEX supports a continued exploration of alternative explanations for the effects of HIEX on appetite regulation.

This study has several limitations. First, the study may be underpowered for measures of FI, but other studies have found this sample size is sufficient for identifying treatment effects on FI (Sim et al., 2014 et al. 2014). However, the time of measuring FI may have been too late. Our previous study showed a potential peak suppression of appetite at 30 min post-HIEX, based on measurements of VAS appetite scores and, appetite and inflammatory biomarkers (Hunschede, Kubant, A., et al., 2017). In the present study, VAS ratings of appetite and hunger were suppressed at 65 min but recovered quickly from 65 to 95 mins after HIEX, at the time of FI measures. Second, females were not included in this study due to financial limitations but this needs to be addressed because several studies have been shown differences in males (Sim et al., 2014) vs. females (Pomerleau et al., 2004) in appetite behaviour after HIEX. Third, the limited
number of blood samples taken in this study may have contributed to failure to detect treatment effects on PYY and GLP-1. We were not able to take blood samples more frequently because the University of Toronto Research Ethics board allows for only 40 mL of blood to be sampled over a 2-hr period in an adolescent study population.
5.5. Conclusion

HIEX reduces subjective appetite but not FI, accompanied by an increase of IL-6 and cortisol and a decrease of active ghrelin. An independent role for IL-6 in appetite suppression was not supported. However, IL-6 was associated with active ghrelin and cortisol, thus potentially mediating appetite via these interactions.

5.6. Conflict of Interest

The authors have no conflicts of interest to report.

5.7. Acknowledgments

We would like to express our gratitude to the parents and children enrolled in the study for their participation; and the volunteers who assisted in the carrying out the study. This study was supported by the Canadian Institute for Health Research (grant/funding no. 490408). Clinical Trial Registry: Clinicaltrials.gov ID: NCT02619461. Authors’ contributions to the manuscript: Sascha Hunschede designed and conducted the experiment, analyzed the data and wrote the research paper. Dr. Ruslan Kubant and Dr. Rajadurai Akilen contributed to the analysis of the data and editing process of the manuscript. G. Harvey Anderson and Scott G. Thomas conceptualized, designed, and supervised the experiment, and had primary responsibility for the final content.
5.8. Tables and Figures

**Table 5.1: Baseline Characteristics**

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<tr>
<td>VO(_{2})peak (mL·Kg(^{-1})·min(^{-1}))</td>
<td>44.3</td>
<td>±</td>
<td>1.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>23.4</td>
<td>±</td>
<td>2.4</td>
</tr>
<tr>
<td>Lean mass (Kg)</td>
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<td>±</td>
<td>2.3</td>
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<tr>
<td>Tanner stage</td>
<td>3.7</td>
<td>±</td>
<td>0.3</td>
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<tr>
<td><strong>Appetite Visual Analog Scores</strong></td>
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<tr>
<td>Appetite (mm)</td>
<td>55.9</td>
<td>±</td>
<td>2.9</td>
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<tr>
<td>DTE (mm)</td>
<td>47.1</td>
<td>±</td>
<td>3.7</td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>45.8</td>
<td>±</td>
<td>3.8</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>32.9</td>
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<td>3.3</td>
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<tr>
<td>PFC (mm)</td>
<td>63.8</td>
<td>±</td>
<td>3.1</td>
</tr>
<tr>
<td>Thirst (mm)</td>
<td>47.1</td>
<td>±</td>
<td>2.8</td>
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<td><strong>Plasma Biomarkers</strong></td>
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<td></td>
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<tr>
<td>Ghrelin (pg/mL)</td>
<td>457.3</td>
<td>±</td>
<td>39.5</td>
</tr>
<tr>
<td>PYY (pg/mL)</td>
<td>66.9</td>
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<td>GLP-1 (pM)</td>
<td>4.4</td>
<td>±</td>
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</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>243.6</td>
<td>±</td>
<td>9.5</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.02</td>
<td>±</td>
<td>0.2</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>5.3</td>
<td>±</td>
<td>0.03</td>
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<tr>
<td>Insulin (pmol/L)</td>
<td>67.8</td>
<td>±</td>
<td>5.6</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.7</td>
<td>±</td>
<td>0.06</td>
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<tr>
<td>IL-6 (pg/mL)</td>
<td>0.96</td>
<td>±</td>
<td>0.08</td>
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Note: DTE, Determination to eat; PFC, Prospective food consumption; PYY, Peptide Tyrosine Tyrosine; GLP-1, Glucagon-like-peptide-1; CRP, C-reactive protein; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor alpha. Values are means ± SEM; n = 15 NW.
Figure 5. 1: Study protocol flow diagram. Note: VAS = Visual analog scale; light grey area = 10 min HIEX bout; dark grey area = 1:30 min rest; blood drop = time point for each blood draw.
Figure 5. 2: Food Intake (A; 100 – 120 min) and Water (B; 0 - 120 min). Letters denote values that are significantly different from control at each time point (3-way ANOVA, Tukey–Kramer post hoc test, p < 0.05). Values are Mean ± SEM, n=15 NW.
Figure 5. 3: Change from baseline in Appetite (A), Prospective Food Consumption (B), Determination to Eat (C), Hunger (D) and Fullness (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean ± SEM, n=15 NW. (*) denotes values that are significantly different between WAEX vs. WARE and (†) denotes values that are significantly different between IBEX vs. IBRE at each TIME point (3-way ANCOVA, Tukey–Kramer post hoc test, p < 0.05). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High-Intensity Exercise; IBEX = Ibuprofen and High-Intensity Exercise.
Figure 5.4: Change from baseline VAS Delta (95-120 min) Appetite (A), PFC (B), DTE (C), Hunger (D) and Fullness (E) scores. Values are Mean ± SEM, n=15 NW, 3-way ANOVA, Tukey–Kramer post hoc test, p < 0.05.
Figure 5.5: Change from baseline plasma levels of Active Ghrelin (A), Peptide YY (B), GLP-1 (C), Blood Glucose (D) and Insulin (E) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean ± SEM, n=15 NW. (*) denotes values that are significantly different between WAEX vs. WARE and (†) denotes values that are significantly different between IBEX vs. IBRE at each TIME point (3-way ANCOVA, Tukey–Kramer post hoc test, *p < 0.05). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High-Intensity Exercise; IBEX = Ibuprofen and High-Intensity Exercise.
Figure 5.6: Change from baseline plasma levels of Interleukin-6 (A), Tumor Necrosis Factor-a (B), Cortisol (C) and C-reactive protein (D) in response to ACT and DRK at 0, 65 and 95 min. Values are Mean ± SEM, n=15 NW. (*) denotes values that are significantly different between WAEX vs. WARE and (†) denotes values that are significantly different between IBEX vs. IBRE at each TIME point (3-way ANCOVA, Tukey-Kramer post hoc test, p < 0.05). Striped blocks represent each 10 min HIEX bout. WARE = Water and Rest; IBRE = Ibuprofen and Rest; WAEX = Water and High-Intensity Exercise; IBEX = Ibuprofen and High-Intensity Exercise.
Chapter 6
ACUTE RESPONSE IN INFLAMMATORY BIOMARKERS AND APPETITE SUPPRESSION AFTER GLUCOSE AND PROTEIN BEVERAGES IN MALE ADOLESCENTS.
Title: Acute Response in Inflammatory Biomarkers and Appetite Suppression after Glucose and Protein Beverages in Male Adolescents.

Running title: Inflammation and Energy Intake with Macronutrient Ingestion in Children.

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Abbreviations: Energy Intake, EI; Glucagon-Like-Peptide-1, GLP-1; Interleukin-6, IL-6; Peptide Tyrosine Tyrosine, PYY; Tumor necrosis factor-alpha, TNF-α; Visual Analog Scales, VAS; Weight Status, WS
Abstract:

Background: Inflammatory biomarkers are found to be associated with food intake regulation in obesity and after HIEX. However, the short-term response of inflammatory biomarkers and their role in satiety and appetite after glucose or protein intake has not been reported.

Hypothesis: Glucose and protein intake will induce changes in Interleukin-6 (IL-6) and Tumour-Necrosis-Factor-α (TNF-α) causing subsequent changes in FI, independent of appetite biomarkers such as Glucagon-Like-Peptide-1 (GLP-1) and insulin.

Objective: To describe the effects of glucose and protein beverages on the acute response of IL-6 and TNF-α, appetite hormones, appetite and FI regulation in male adolescents.

Results: FI, appetite and inflammatory biomarkers were not affected by glucose or protein. GLP-1 (P<0.001) and insulin (P<0.001) were increased by the caloric beverages while ghrelin was decreased (P<0.001). Mean post-beverage IL-6 correlated (r=-0.377, p<0.004) with meal food intake. GLP-1 was associated with decreased appetite (r=-0.535; p<0.001) and FI (r=-0.41; p=0.002) and positively with increased insulin (r=0.45; p<0.001). Insulin was also negatively associated with active ghrelin (r=-0.358; p=0.005) and FI (kcal) (r=-0.276; p=0.03).

Conclusion: Acute responses in IL-6 and TNF-α, in contrast to appetite hormones, are not affected by ingestion of either glucose or protein beverages. However, the negative relationship between IL-6 prior to the meal with FI may indicate that IL-6 is a determining factor in FI regulation.
6.1. Introduction:

The response of gut peptides (active ghrelin, PYY, GLP-1 and CCK) following the ingestion of food (Prinz & Stengel, 2017) and its macronutrients, glucose (GLU) (Shiiya et al., 2002), protein (PRO) (Caron, Domenger, Dhulster, Ravallec, & Cudennec, 2017) and fat (Little, Horowitz, & Feinle-Bisset, 2007) has received extensive study. Similarly, the inflammatory cytokines (IL-6, TNF-α and possibly CRP) are associated with inhibition of feeding behaviour, as shown by decreased food intake (FI) after their direct intraventricular injection in the central nervous system, or as a result of the administration of bacterial lipopolysaccharide (LPS), in experimental animal models (Benrick et al., 2009; Morley, Levine, & Kneip, 1981; Morley et al., 2006; Ropelle et al., 2010; Shizuya et al., 1997). The resulting inflammatory anorexia is often expressed as reduced meal frequency and size (Langhans et al., 1993), an aversion for novel foods or sucrose (Bauer, Weingarten, Senn, & Langhans, 1995; Goehler et al., 1995; Langhans et al., 1990; Tazi et al., 1988; Weingarten, Senn, & Langhans, 1993), and inhibition of food-motivated behaviour (Bret-Dibat, Bluthe, Kent, Kelley, & Dantzer, 1995). Furthermore, our previous research showed that short-term appetite suppression following HIEX in children was associated with reduced appetite, increased plasma IL-6, cortisol and decreased active ghrelin and blood glucose (Hunschede, Kubant, Akilen, et al., 2017; Hunschede, Schwartz, Kubant, Thomas, & Anderson, 2018).

Inflammation and the accompanying suppression of appetite have also been proposed as beneficial for increasing survival rates in the presence of infection or other diseases (Wing & Young, 1980). This may occur due to energy repartitioning in support of immune responses by reducing activities such as food-seeking and digestion (Exton, 1997; Hart, 1988) as well as by a
decrease in micronutrient availability needed for bacterial proliferation (Weinberg, 1984). However, high levels of chronic inflammation are detrimental to survival. Patients with HIV and cancer often display chronic inflammation and drastic weight-loss and increased mortality, also accompanied by a general loss of appetite (Laviano et al., 2003; Tisdale, 2009).

In contrast, elevated, albeit to a much lesser degree, higher inflammatory biomarkers and altered appetite hormones are found in a fasted state in the presence of the metabolic syndrome (Chedraui et al., 2014; Indulekha et al., 2011; Weiss et al., 2013), arising from excess FI and the accumulation of adipose tissue. The cytokines, interleukin-6 (IL-6), tumour-necrosis-factor-α (TNF-α) and C-reactive protein (CRP), are upregulated by and expressed due to increased macrophages in adipose tissue (Hotamisligil et al., 1993; Jung & Choi, 2014; Ouchi et al., 2011), causing low-grade systemic inflammation and subsequent insulin resistance (Kern et al., 2001). When fasting, overweight (OW)/obese (OB) individuals also have higher leptin but lower ghrelin, (Carlson, Turpin, Wiebke, Hunt, & Adams, 2009) and glucagon-like-peptide-1 (GLP-1) levels (Ahmed et al., 2017) but no differences in peptide YY (PYY) (Cahill et al., 2014) when compared to NW individuals. Post-prandially, leptin (Carlson et al., 2009), PYY (Prinz & Stengel, 2017) and GLP-1 (Lean & Malkova, 2016) is higher (Carlson et al., 2009), and ghrelin (Carlson et al., 2009) lower in OB when compared with normal-weight (NW), indicating dysfunctional appetite regulation and possible hypothalamic inflammation. Inflammation in the brain impairs appetite regulation, energy homeostasis and induces hyperphagia, most likely due to decreased insulin and leptin sensitivity (Timper et al., 2017). IL-6 can be produced by macrophages in the brain and potentially mediates appetite and FI by bypassing the blood-brain barrier and attach to receptors.
in the hypothalamus, affecting appetite, FI and even food preferences (Benrick et al., 2009; Ropelle et al., 2010; Shizuya et al., 1997).

Our previous research has provided evidence for a relationship between short-term inflammatory responses mediated by high-intensity-exercise (HIEX) and suppression of appetite (Hunschede, Kubant, Akilen, et al., 2017; Hunschede et al., 2018). However, the ingestion of food represents an entirely physiological different process when compared to HIEX, and the effects of macronutrient ingestion on inflammatory markers and their subsequent effect on later appetite and FI regulation has not been reported.

The hypothesis of this study was that IL-6 and TNF-α respond acutely to glucose and protein macronutrient preloads in male adolescents and play a role in appetite and FI independent of appetite hormones.

The objective is to describe the effects of glucose and protein preloads on biomarkers of inflammation, appetite hormones and their subsequent effect on appetite and FI regulation in male adolescents.
6.2. Methods:

6.2.1. Participants:

A sub-sample of 20 participants were analysed from a previous study that investigated the effects of macronutrient preloads on testosterone response and its relationship with appetite and FI in 12-18-year-old adolescent males (A. Schwartz et al., 2019). Healthy adolescent males were recruited via a print ad in the local newspaper. Using sample size calculations based on our previous study (Hunschede, Kubant, Akilen, et al., 2017) we determined that a sample size 20 would be sufficient to detect a 10% response with a power of 0.80 and an \( \alpha \) of <0.05 in appetite hormones and inflammatory markers and a 20% response in appetite and a 15% response in appetite and FI. Body-weight percentiles were defined using the Centre for Disease Control BMI charts; participants were included if they displayed were within 15\(^{th}\)-99\(^{th}\) BW percentile (Flegal et al., 2002). Participants were excluded if they exhibited one of the following characteristics: prematurity, chronic illness or were taking any medications known to affect GLU homeostasis, appetite or pubertal development. Height (cm) and weight (Kg) were measured using a stadiometer and digital scale, while body composition was determined by bioelectrical impedance analysis (RJL Systems BIA, 101Q) using the Horlick equation (Horlick et al., 2002). The study was approved by the University of Toronto Research and Ethics Board for Humans.

6.2.2. Experimental Design and Protocol:

The study employed a two-level mixed factorial (TREATMENT x TIME) repeated measures randomized design. All participants completed three treatment (TRT) sessions in a randomized order: (i) 1g bodyweight of GLU monohydrate (BioShop Canada Inc., Burlington, Ontario, Canada)
with 500mL water (Danone Crystal Springs, Quebec City, QC, Canada); (ii) 1g per kg of plain whey-PRO isolate (BiPro USA., Eden Prairie, Minnesota, U.S.A) with 500mL water, and (iii) a 500mL non-caloric control (CON) drink. All drinks were flavoured with 1.5mL of chocolate extract (Vanilla Food Company, Markham, Ontario, Canada) to standardize the flavour. The control and whey PRO drink were also flavoured with 0.2g sucralose (Tate & Lyle, Stoney Creek, Ontario, Canada) to match the sweetness of the GLU beverage.

Experimental sessions were conducted at the University of Toronto FitzGerald building on weekends beginning between 8 and 9 am after a 12-h overnight fast, at the same time and day of the week, one week apart. Children were asked to refrain from exercise at least 24-h before the experimental session and parents were asked to encourage their children to drink water 1-h before the scheduled session, to refrain from physical activity, and to maintain the same dietary patterns the evening before each test. Upon arrival, participants were asked to complete the baseline VAS questionnaire followed by the insertion of an IV catheter in the cubital fossa vein. After the collection of the first blood sample, the timer was started at 0 min, and participants were given the beverage and had 10 min to consume it. Blood samples were drawn at baseline (0 min), then at 15, 30 and 60 min.

Figure 6.1

6.2.3. Food Intake

At 60 min, an ad libitum pizza meal was provided to the participants. Based on subject preferences determined during screening, two varieties of Deep’N’Delicious 5-inch-diameter
pizza were provided for consumption; pepperoni and three-cheese pizzas (McCain Canada Ltd., Florenceville, Ontario, Canada). Pepperoni pizza (87g) contained 9g of PRO, 6g of fat and 23g of carbohydrates for the total energy content of 180 kcal. Each three-cheese pizza (81g) contained 10g of PRO, 6g of fat and 23g of carbohydrate for the total energy content of 180 kcal. The cooked pizzas were cut and weighed into four equal pieces before serving, and the amount left after the meal was subtracted from the initial weight and converted to kcal per kg body-weight to provide a measure of FI. These pizzas lack an outer crust resulting in a uniform energy content thus mitigating the prospect for the subject to eat the energy-dense filling and leave the outside crust of the pizza. Water consumption was determined by weight.

6.2.3.1. Visual Analog Scales

Motivation to eat visual analog scales (VAS) were employed to assess subjective appetite, which was calculated based on the following questions: (1) How strong is your desire to eat? (“very weak” to “very strong”), (2) How hungry do you feel? (“not hungry at all” to “as hungry as I have 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”). Participants were instructed to read each question and place an “X” along a 100-mm line depending on how they felt at the current moment. Visual analog scale questionnaires were administered at baseline (0 min), 10, 30, 60 and 80 min after the start of each session (Bellissimo et al., 2008; Bellissimo, Pencharz, Thomas, & Anderson, 2007).
6.2.3.2. Blood Collection and Plasma Sample Processing

Blood samples were collected into pre-chilled 10 mL BD Vacutainer™ (BD Diagnostics, Sparks, MD, USA) blood collection tubes containing spray-dried K2EDTA anticoagulant, and a proprietary cocktail of protease inhibitors [e.g. DPP-IV (R-3-Amino-1-{3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazol[4,3-a]pyrazin-7-yl]-4(2,4,5trifluorphenyl)butan-1-on), AEBSF (4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride) and aprotinin (Trasylol)] to prevent the proteolytic breakdown of hormones. Immediately after collection, plasma was separated by centrifugation (Eppendorf, Hamburg, Germany for 15 min at 2000 relative centrifugal force at 4°C, then aliquoted into 2 mL Eppendorf (Eppendorf, Hamburg, Germany) tubes and stored at -80°C for later analyses. In addition, to enhance the active ghrelin stability in blood samples, 200 µL 1 N HCl/ml was added to the plasma.

6.2.3.3. Biochemical Measurements

All analyses were performed in the Department of Nutritional Sciences at the University of Toronto. The biological active form of ghrelin, GLP-1, and Insulin [i.e. GLP-1 (7-36 amide)] were analyzed by using commercial ELISA kits purchased from Millipore (Millipore, Billerica, MA, USA): (Active ghrelin cat. #EZGRA-88K, Sensitivity: 8pg/mL, Range: 25-2000 pg/mL; GLP-1 cat. #EGLP-35K, Sensitivity: 2pM, Range: 2-100 pM; Insulin cat. #EZHI-14K, Sensitivity: 2 μU/mL, Range: 2–200 μU/mL). Interleukin-6 and TNF-α were analyzed using sandwich ELISA kits from R&D systems (R&D Systems, Minneapolis, MN, USA); (IL-6 cat. #HS600B, Sensitivity: 0.11 pg/mL, Range: 0.156–10 pg/mL; TNF-α cat. #HSTA00D, Sensitivity: 0.191 pg/mL, Range: 0.5 – 32 pg/mL). For all assays, intra-CV was <4%, and Inter-CV was <8%.
6.2.3.4. Statistical Analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, N.C., USA). The randomization schedule was generated with the SAS PROC PLAN procedure. All data were tested for normality using the PROC UNIVARIATE procedure. To analyze the main effects of TRT (CON vs. GLU vs. PRO), time and their interaction on VAS scores and blood biomarker change from baseline, a 2-factor ANCOVA was performed (by PROC MIXED procedure) followed by Tukey–Kramer’s post-hoc test with baseline (0 min) as a covariate. One factor ANOVA was used, followed by Tukey-Kramer’s post-hoc test to compare the effect of treatments at each time of measurement when an interaction between TRT and time was found. Pearson correlation analysis (Spearman’s rank coefficients for non-normal data) was conducted to test associations between absolute means of each biomarker and with VAS scores and FI. All data are presented as absolute mean ± SEM. Statistical significance was declared at $P < 0.05$. 


6.3. Results:

Participant characteristics are displayed in Table 6.1.

6.3.1.1. Food Intake

FI (kcal/kg) was not affected by TRT (P = 0.799). Participants ate 19.2 ± 1.5 kcal/kg with CON, 17.9 ± 1.5 kcal/kg with GLU, and 18.3 ± 1.2 kcal/kg with PRO. No interaction among the factors was found.

6.3.1.2. Visual Analog Appetite Scales:

Appetite was not affected by TRT (P = 0.472) but decreased over TIME (p < 0.001) from baseline to 15 min, and then recovered to 60 min. DTE was not affected by TRT (P = 0.914) but increased steadily over TIME (P = 0.017). Hunger was also not affected by TRT (P = 0.743) but increased over TIME (P = 0.002). Fullness was not affected by TRT (P = 0.308) but increased over TIME (P < 0.001). PFC was not affected by TRT (P = 0.141) but increased over TIME (P = 0.006). No other interactions were found.

Figure 6.2

6.3.1.3. Inflammatory Biomarkers:

IL-6 was not affected by TRT (P = 0.5) or TIME (P = 0.06). TNF-α was also not affected by TRT (P=0.729) or TIME (P = 0.402). No other main effects or interactions were found.

Figure 6.3
6.3.1.4. Appetite Biomarkers:

Active ghrelin was affected by TRT (P < 0.001), TIME (P = 0.01), with a TRT and TIME interaction (P = 0.033). Both GLU and PRO decreased ghrelin at 60 min compared with control. The interaction was explained by a decrease of active ghrelin after GLU at 15 min (P = 0.004) and 30 min (P < 0.001) but not PRO compared with control. GLP-1 was affected by TRT (P < 0.001) and TIME (P < 0.001) and a TRT and TIME interaction (P < 0.001). Both GLU and PRO beverages increased GLP-1 at 60 min. The interaction effect between TRT and TIME on GLP-1 is explained by its immediate rise at 30 min after GLU (P < 0.001) and PRO (P = 0.001) but not CON. Insulin was increased by TRT (P < 0.001) and TIME (P < 0.001) and affected by an interaction of TRT and TIME (P < 0.001). The insulin TRT and TIME effect is explained by the much larger increase in plasma insulin with GLU than after PRO.

Figure 6.4

6.3.2. Correlations:

Absolute means of IL-6 prior to the lunch meal in the pooled data was negatively correlated with FI (kcal/kg) (r = -0.377; p = 0.004), and TNF-α (r = 0.329; p = 0.011). GLP-1 was positively associated with Fullness (r = 0.478; p < 0.001) and insulin (r = 0.45; p < 0.001) and negatively with appetite (r = -0.538; p < 0.001), DTE (r = -0.411; p < 0.001), hunger (r = -0.49; p < 0.001) and PFC (r = -0.329; p = 0.015) and FI (kcal/kg) (r = -0.41; p = 0.002). Insulin was negatively associated with active ghrelin (r = -0.358; p = 0.005) and FI (kcal/kg) (r = -0.276; p = 0.03). FI (kcal/kg) was also positively correlated with appetite (r = 0.387; p = 0.002) and DTE (r = 0.382; p = 0.003).
6.4. Discussion:

The results did not support our primary hypothesis. IL-6 and TNF-α did not respond acutely after GLU and PRO ingestion and FI was not affected. However, the post-beverage mean response in IL-6 was strongly associated with reduced mealtime FI in the pooled sample, thus supporting a role for IL-6 in FI regulation. However, the post-beverage insulin and GLP-1 response to treatments were also negatively associated with meal-time FI. Thus an independent effect of IL-6 was not shown.

IL-6 and TNF-α were not affected by either GLU or PRO ingestion, and no correlation was found between either IL-6 or TNF-α with appetite, FI or appetite regulatory hormones, which suggests that there is no physiological significance of the acute food-induced responses in IL-6 and TNF-α. On the other hand, the inverse association between post-treatment IL-6 and FI and 60 mins in the pooled data suggests it does play a role in FI regulation. As shown in Figure 6.5 there is no distinction in the spread of the data based on GLU or PRO treatment. Interpretation may be that the sample size too small for treatment effect, or that chronic systemic inflammation in the individual is the determinant of appetite control and the relationship between IL-6 and FI. Sustained low-grade inflammation and an above-average appetite are commonly found in OB individuals and children (Visser, Bouter, McQuillan, Wener, & Harris, 2001). Since FI is increased in OB, it seems counterintuitive that the acute inflammation associated with many illnesses and HIEX as shown by our previous research (Hunschede, Kubant, Akilen, et al., 2017; Hunschede et al., 2018), suppresses appetite. However, emerging evidences show that chronic inflammation induced by a high-fat diet or endotoxins induce activation of non-neuronal cells such as astrocytes and microglia to produce inflammatory reactions in the hypothalamus and disrupt its
normal function (Feng et al., 2017; Valdearcos et al., 2017; Yulyaningsih et al., 2017), eventually causing a dysregulation of appetite rather than appetite control, leading to OW/OB individuals being less hungry in a fasted state and less full after consuming a meal (Chapter 4). Even, after weight-loss, this hypothalamic inflammation persists, putting OB individuals at risk for re-gaining weight (Sumithran et al., 2011).

No response in IL-6 and TNF-α following GLU and PRO beverages contrasts with our previous study that showed an increase in IL-6 associated with a decreased appetite after HIEX (Hunschede, Kubant, Akilen, et al., 2017). However, this can be explained by two completely different and even opposing processes occurring after HIEX and the ingestion/digestion of food. During FI, splanchnic blood flow is directed to the gut to facilitate the secretion of gut peptides and peristalsis of the stomach to begin the chemical breakdown of proteins. Shortly (5 min) after FI, mesenteric blood flow is increased by 60% and 113% after 60 minutes. This occurred while cardiac output was only increased by 12-22%, suggesting a redistribution of blood flow to the intestines, rather than an overall increase in blood flow (Norryd, Denker, Lunderquist, Olin, & Tylen, 1975). Blood flow in the celiac artery also peaks rapidly at 38%–60% above fasting levels (Aldoori, Qamar, Read, & Williamson, 1985; Someya et al., 2008), while blood flow in the superior mesenteric artery increases 1.5- to 3.5-fold, 5–60 min after a meal (Qamar & Read, 1988; Sidery et al., 1991; Someya et al., 2008). However, this process is reversed with HIEX. During HIEX, blood vessels are dilated to increase blood flow to the working extremities and contracting muscle (Joyner & Casey, 2015). Blood flow during cycling increased mesenteric, coeliac and splanchnic resistance by 76%, 165% and 126%, respectively, and reduced corresponding blood flows by 32%, 50% and 43% (Perko et al., 1998). Another study showed that HIEX at 70% of \( VO_2 \text{max} \) decreased
blood flow by 80% in the portal vein after 60 min of cycling (Rehrer et al., 2001) and by 43% in the superior mesenteric artery subsequently to 30 min of treadmill running (Qamar & Read, 1987). Our hypothesis then follows the appetite suppressing effect during and after HIEX is controlled by other mechanisms, such as inflammatory processes when compared to the satiation and satiety effects seen during food ingestion and digestion. The lack of effect of the treatments on FI and appetite with PRO ingestion is surprising because previous studies have shown a decrease in appetite and FI after PRO ingestion in children as well as adults (Akhavan, Luhovyy, & Anderson, 2010; Akhavan et al., 2014; G. H. Anderson et al., 2011; G. H. Anderson & Moore, 2004; G. H. Anderson, Tecimer, Shah, & Zafar, 2004; Bellissimo et al., 2008) after similar doses.

The study has several limitations. First, the sample size for measures of FI and appetite may have been too small to detect inflammatory changes with macronutrient intake. The sample size of this study has been based on our previous HIEX experiments. Through this study it also becomes apparent that HIEX is a much stronger stimulus for IL-6 and TNF-α, compared to macronutrient intake. Second, the period of 60 min post drink and the ad libitum meal may have been too long to induce a change in FI with the 1.0 g BW preload. FI is decreased when measuring 30 min after a 1.0 g BW GLU preload, but not at 60 min (Bellissimo et al., 2008). Finally, female participants were not included in the study due to financial limitations. However, females have several different appetite biomarkers compared to men and also often express appetite differently (Bedard et al., 2015; Williams, Wood, Collins, Morgan, & Callister, 2016). This could potentially imply differences in appetite regulation.
6.5. Conclusion

Acute responses in IL-6 and TNF-α, in contrast to appetite hormones, are not affected by ingestion of either GLU or PRO beverages. However, the negative relationship between IL-6 before the meal with FI may indicate that IL-6 is a determining factor in FI regulation.

6.6. Conflict of Interest

The authors have no conflicts of interest to report.

6.7. Acknowledgments

We want to express our gratitude Mr. Alexander Schwarz for allowing us to use his samples to conduct this secondary analysis. This study was supported by the Canadian Institute for Health Research (grant/funding no. 490408). Clinical Trial Registry: Clinicaltrials.gov ID: NCT03412136. Authors’ contributions to the manuscript: Sascha Hunschede designed and conducted the experiment, analysed the data and wrote the research paper. Dr. Ruslan Kubant contributed to the analysis of the data and editing process of the manuscript. G. Harvey Anderson conceptualized, designed, and supervised the experiment, and had primary responsibility for the final content.
6.8. Tables and Figures

Table 6.1: Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.0 ± 4.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.6 ± 3.2</td>
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<tr>
<td>BMI (kg·m(^2))</td>
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<td>BMI percentile</td>
<td>68.0 ± 8.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16.3 ± 2.3</td>
</tr>
</tbody>
</table>

*BMI, body mass index; Values are means ± SEM; n = 20
Figure 6. 1: Study protocol flow diagram. Note: VAS = Visual analog scale; blood drop indicates blood draw.
Figure 6.2: *Change from baseline in Appetite (A), Determination to Eat (B), Hunger (C), Fullness (D) and Prospective Food Consumption (E) VAS in response to TRT at 0, 15, 30, and 60 min (2-way ANCOVA with baseline as covariate, Tukey–Kramer post hoc test, p < 0.05). Values are Mean ± SEM, n=20. Letters denote significant differences at each time point.*
Figure 6. 3: Change from baseline plasma levels of IL-6 (A) and TNF-α (B) in response to TRT at 0, 15, 30, and 60 min (2-way ANCOVA with baseline as a covariate, Tukey–Kramer post-hoc test, p < 0.05). Values are Mean ± SEM, n=20. Note: Letters denote significant differences at each time point.
Figure 6.4: Change from baseline plasma levels of Active Ghrelin (A), Glucagon-like-peptide 1 (B) and Insulin in response to TRT at 0, 15, 30, and 60 min (2-way ANCOVA with baseline as a covariate, Tukey–Kramer post hoc test, p < 0.05). Values are Mean ± SEM, n=20. Note: Letters denote significant differences at each time point.
Figure 6.5: Pearson correlation between FI and IL-6. CON, GLU, and PRO are shown in grey, red and blue respectively.
7.1. General Discussion

This thesis is a report of novel studies identifying a potential new area of investigation in FI regulation. Prior to this research, the role of inflammation in appetite control has had little examination. This Ph.D. project was designed in three parts. The first experiment was an exploratory approach to investigate the effects of HIEX on inflammatory biomarkers that could potentially affect appetite and FI in children. The second experiment utilized findings from the first experiment with a more focused design and utilized an inhibitor (IBU) of inflammatory response to further understand the role of inflammation. The third experiment complemented the first two studies by comparing the effects of HIEX with macronutrient ingestion. All experiments were conducted in a randomized, when possible blinded, crossover design. By using a crossover design, we minimized the effect of inter-individual differences in appetite regulation, which can be quite large in humans (J. A. King et al., 2017).

These studies further our understanding of the effect of acute inflammatory responses to HIEX and macronutrients in the regulation of appetite and FI. The primary hypothesis that acute responses in IL-6 play a role in the regulation of appetite and FI after HIEX and macronutrient preload was partially supported. HIEX consistently induced increased levels of IL-6 (Chapter 4 and 5). In contrast, macronutrient preloads did not affect IL-6 and TNF-α, although mean absolute values for IL-6 post-treatment correlated strongly with next meal intake in the pooled data.
(Chapter 6). However, concurrent increases in appetite-regulating hormones in response to treatments were found in all studies, leaving uncertain an independent effect of IL-6.

Our results indicated a role of IL-6 with HIEX and appetite regulation, but we could not establish IL-6 as an independent factor. IL-6 consistently increased with HIEX (Chapter 4 and 5) when compared to rest, with no effect of WS. These results in children are consistent with research in adults showing that IL-6 plasma levels were increased up to 30-fold after strenuous EX (Waskiewicz et al., 2012), often accompanied by a loss of appetite, which can also be observed with overtraining (L. L. Smith, 2000). However, the effects of HIEX in children were modest as IL-6 plasma levels were only increased 1.5 times (Chapter 4), with a temporary appetite suppression immediately after HIEX. Nevertheless, these are the first results to show a positive association between IL-6 and appetite (Chapter 4) and the negative relationship between IL-6 and FI (Chapter 6).

Furthermore, IL-6 has been shown to stimulate GLP-1 secretion and increase PYY expression in other studies. However, GLP-1 and PYY were not changed by HIEX, and therefore IL-6 did not mediate appetite via these interactions. It is possible that a stronger IL-6 (>100 fold from baseline) response is required to initiate changes in GLP-1 and PYY (Ellingsgaard et al., 2011). IL-6 was also associated with active ghrelin and cortisol (Chapter 5) potentially mediating its appetite-suppressing effects directly but also via these interactions. In our glucose and protein study (Chapter 6), we saw a correlation between baseline levels of IL-6 and FI indicating that daily IL-6 levels might be involved in total FI, independent of the glucose and protein pre-loads. Aside from peripheral effects, IL-6 can also pass the blood-brain barrier and directly interact with the same neuronal circuits that are affected by appetite hormones (Pazos, Lima, Casanueva, Dieguez,
& Garcia, 2013; Schele et al., 2013; Shirazi et al., 2013). These results indicate a role of IL-6 in FI and appetite regulation, but the exact mechanisms need to be studied further.

Active ghrelin showed several correlations with other biomarkers that could have affect appetite behaviour as well. Active ghrelin was decreased across all three studies, with HIEX or macronutrient ingestion, potentially decreasing appetite when suppressed; however, the time course of active ghrelin did not always match the time course of appetite VAS, making its role uncertain. Active ghrelin is the only hormone known to signal hunger. Ghrelin in its active form decreased in children after HIEX, consistent with the response in adults (Broom et al., 2007), potentially mediating the appetite suppression after HIEX (Chapter 4 and 5). Furthermore, the suppression of active ghrelin was only short-lived until the end of exercise and recovered quickly in both HIEX experiments. In experiment 1 the time-course of active ghrelin did not match the time course of appetite. This disassociation of active ghrelin with appetite in exercise studies has also been demonstrated previously (Broom et al., 2017). In experiment 2, active ghrelin was also decreased and matched the time-course of appetite. However, in both experiments no correlation with appetite was found. One possible explanation why HIEX could decrease active ghrelin is the accumulation of lactate. Lactate accumulates when lactate production exceeds its clearance (Ohkuwa et al., 2009). High physiological concentrations of lactate have shown to suppress the secretory function of ghrelin producing gastric cells (Engelstoft et al., 2013). This may explain how active ghrelin is decreased with exercise at high intensities, possibly exerting its effects on appetite. Active ghrelin was also suppressed after consumption of GLU and PRO beverages (Chapter 6), but neither FI or appetite was affected. Although correlations are seldom
show and active ghrelin and appetite frequently disassociated, the suppression of appetite or FI is often conceded to the decrease in active ghrelin with HIEX (Broom et al., 2007).

CRP and cortisol did not show any associations with appetite or FI. CRP was measured in experiment 1, which limited the range of data derived for CRP responses (Chapter 4). Cortisol was measured in experiment 1 and experiment 2 (Chapter 4 and 5), but both were not measured in experiment 3 (Chapter 6) due to low plasma volumes and the absence of an OW/OB group in the design. CRP did not respond to HIEX which is not surprising as CRP is known to be a tonic marker for inflammation and is often used to compare systemic levels of inflammation in NW and OW/OB individuals (Ellulu, Patimah, Khaza’ai, Rahmat, & Abed, 2017). In both studies, plasma cortisol increased significantly shortly after HIEX, when compared to rest, and declined as soon as the HIEX session was concluded. Cortisol is the typical stress biomarker and often associated with metabolic syndrome and diabetes (Adam et al., 2010). Cortisol has also been shown to predict future weight gain (Chao, Jastreboff, White, Grilo, & Sinha, 2017) and affect appetite and FI in the short-term (Lawson et al., 2011). The exact mechanisms how cortisol can affect appetite, directly and indirectly, remain to be elucidated. In addition, we did not see an association with appetite or FI. Cortisol also showed a strong association with active ghrelin (Chapter 4 and 5) as well as IL-6 (Chapter 4). However, many studies fail to find a correlated response, but this is consistent with the observation that no single biomarker of appetite and FI has been identified to the present time (de Graaf et al., 2004).

Results from our three studies make it difficult to establish a role for TNF-α in appetite and FI regulation with HIEX or GLU and PRO intake. In the first HIEX study (Chapter 4), TNF-α decreased from baseline and recovered by the end of the experiment. However, in experiment
HIEX did not affect TNF-α levels (Chapter 5), similarly to PRO and GLU (Chapter 6) intake. A few studies showed an increase of TNF-α with HIEX (Bernecker et al., 2013; Northoff, Weinstock, & Berg, 1994); however, the relationship with HIEX is not as strong as the one with IL-6 and HIEX (Pedersen & Toft, 2000). In contrast to IL-6, TNF-α was not correlated with the appetite of FI in either of the studies. These inconsistent results may be, in part, due to the substantial diurnal rhythm changes in TNF-α. Recent evidence suggests that the circadian clock not only regulates immune responses but also is regulated by the components of immune system, in a bidirectional communication (Arjona & Sarkar, 2005; Cavadini et al., 2007; Nakao, 2014). Although several immunomodulatory cytokines play an essential role, TNFα functions as bridging element between the circadian clock and the immune system (Ertosun, Kocak, & Ozes, 2019).

In contrast to ghrelin, PYY and GLP-1 did not respond consistently to HIEX. In the first study, there was no difference in PYY or GLP-1 after HIEX (Chapter 4). The present results in children are consistent with studies in adults showing that GLP-1 and PYY do not show consistent behaviour, increasing in some studies while others report no effect or even a decrease of PYY and GLP-1. In one study, an acute bout of HIEX lowered active ghrelin and increased PYY as well as GLP-1 (Deighton et al., 2013; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). In contrast, HIEX has been reported to increase active ghrelin (Larson-Meyer et al., 2012), decrease GLP-1 (Unick et al., 2010) and do not affect PYY (Larson-Meyer et al., 2012). Thus, as concluded in a meta-analysis of current studies acute bouts of EX may have only slight to moderate effects on appetite-regulating hormones (Schubert et al., 2014). In our third study, PYY was not measured, but GLP-1 increased with GLU and PRO intake, which is consistent with the literature on macronutrient intake (Giezenaar et al., 2017;
Hiroyoshi et al., 1999). Furthermore, after consumption of GLU and PRO beverages, GLP-1 was strongly associated with FI, and appetite (Chapter 6).

Insulin behaved somewhat inconsistently in our studies. It was increased with PRO and GLU in our third study (Chapter 6). While we saw a constant decrease of Insulin over time without an effect of IBU or HIEX in our second study (Chapter 5), we saw an increase of insulin in NW but not OW/OB with HIEX compared to rest in our first study (Chapter 4). The literature also reported that insulin concentrations decreased in response to moderate EX and HIEX (Ribeiro et al., 2004) due to an increase of insulin sensitivity and increased GLU uptake in the muscle, without additional insulin secretion (Goodyear & Kahn, 1998). The adequate regulation of insulin during HIEX is crucial to prevent hypoglycemia; however, sometimes insulin production also needs to be increased in some cases to increase GLU uptake in the muscle to match the energy demand during HIEX (Marliss & Vranic, 2002). This may explain the varying results in our two studies. In our first study, we enrolled NW and OW/OB participants, and to ensure compliance to our HIEX protocol we set the intensity at 70% VO2max (Chapter 4). For our second study (Chapter 5) we included only NW participants and set the intensity at 75% VO2max. Although the difference in intensity was only 5%, this may have caused a shift in insulin secretion to ensure a sufficient supply of GLU during the higher EX intensity. Insulin has been implicated in the regulation of appetite for decades (Rodin, 1985). We found a correlation between insulin and FI (Chapter 6) and appetite (Chapter 5), thus indicating a role of insulin in appetite and FI regulation.

The modern idea of appetite regulation is based on the hypothesis that the brain can detect alterations in energy stores and generate metabolic and behavioural responses to maintain EB. Eating behaviour is motivated by hunger, cravings and hedonic sensations, designed
to control energy homeostasis. The same idea has been utilized to explain appetite sensations changes in FI with HIEX. However, as explained in the previous chapter, the ingestion of food and EX are two completely different processes.

FI and HIEX represent two distinct and opposing physiologic processes. With HIEX sympathetic nervous system activity is increased and the blood flow is redistributed away from the GI tract, towards the skeletal muscles (Ikeda et al., 2010), and during FI blood flow is redistributed from the periphery towards the GI tract (Madsen, Sondergaard, & Moller, 2006). During FI, splanchnic blood flow is directed to the gut to facilitate the secretion of gut peptides and peristalsis of the stomach to begin the chemical breakdown of proteins. Shortly (5 min) after FI, mesenteric blood flow is increased by 60% and 113% after 60 minutes. This occurred while cardiac output was only increased by 12-22%, suggesting a redistribution of blood flow to the intestines, rather than an overall increase in blood flow (Norryd et al., 1975). Blood flow in the celiac artery also peaks rapidly at 38%–60% above fasting levels (Aldoori et al., 1985), while blood flow in the superior mesenteric artery increases 1.5- to 3.5-fold, 5–60 min after a meal (Qamar & Read, 1988; Sidery et al., 1991; Someya et al., 2008) (Chapter 5 and 6). In contrast, during HIEX splanchnic blood flow is decreased and reallocated from the gut to contracting skeletal muscle to increase oxygen supply (Eriksen & Waaler, 1994). HIEX at 70% VO\textsubscript{2}max decreased blood flow in the portal vein by 80% subsequently to 60 min of cycling at (Rehrer et al., 2001) and by 43% after in the superior mesenteric artery after 30 min of treadmill running (Qamar & Read, 1987). This contrast in blood flow after HIEX supports a continued exploration of alternative explanations for the effects of HIEX on appetite regulation.
It is reasonable to deduce that this redistribution of blood flow is also accompanied by an increase in metabolic activity and biomarker secretion in the perfused tissue, in turn secreting various metabolites that can affect appetite. When food is ingested, and blood flow is directed to the gut, gut peptides could be released to a greater extent or even triggered to be secreted by gastric activation. With EX, splanchnic blood flow is redistributed from the GI tract to the skeletal muscle. There is a consistent increase of myokines which mediate the decrease in appetite during and shortly after EX (Hunschede et al., 2016). Ghrelin is also consistently decreased with HIEX, potentially reflecting the redistribution of splanchnic blood flow, decreasing appetite. The role of other appetite hormones such as GLP-1 and PYY is less clear, as their behaviour with HIEX is somewhat inconsistent.

A hypothetical model to show FI and EX could mediate splanchnic blood flow and affect subsequent appetite as shown by these three experiments is captured in Figure 8.1.
Figure 7. 1: Main mechanism engaged in appetite regulation with FI and EX. Blood flow is redirected to the GI tract with FI, causing the subsequent release of gut peptides and signalling the cessation of hunger and an increase in satiety and satiation. With EX blood flow is directed to the skeletal muscles which release IL-6 and in turn cause feelings of nausea, food preferences and a decrease in overall appetite.
7.2. Limitations:

This study had several limitations described in the following section. First, the study design. In our study, the intensity was limited to either 70% VO$_2$max or 75% VO$_2$max at 30 min of HIEX. Some research suggests a disconnect between EX-induced energy expenditure and EI, especially in children and adolescents (Thivel & Chaput, 2014). Contributing to this may be a larger intra- and inter-individual variation in appetite in response to EX (J. A. King et al., 2017). Therefore, a more significant stimulus to induce changes in FI in experiment 2 may have been needed (Chapter 5). It is possible that we “missed” a certain intensity and duration threshold in order to induce suppression of appetite as well as FI. We chose 70-75% VO$_2$max to ensure manageble attrition rates, which is especially crucial while working with children.

Furthermore, time to next meal was also a factor that may have contributed to missing the time point of FI suppression. Appetite, in our two studies did not behave consistently. In experiment 1 (Chapter 4) we saw a steady increase in appetite, but lower values with HIEX when compared to rest. In experiment 2 (Chapter 5), appetite with HIEX briefly fell below baseline, shortly after HIEX and then recovered quickly to baseline while resting appetite values increased steadily. The appetite suppression after HIEX is transient, and therefore in the first study we determined the time point where the delta between resting and HIEX appetite values was at its highest point. We found that 30 min post-exercise was the ideal point to initiate the lunch meal. Looking at our second experiment (Chapter 5), we see suppression of appetite only after the cessation of HIEX which then recovers to baseline. Therefore, it may have been possible that we “missed” an FI response because the time between HIEX and the initiation of the lunch meal was too long. Exercise mode may also play an additional effect on appetite behaviour post-exercise.
We used a recumbent bicycle for our HIEX sessions. Cycling predominately recruits muscle groups in the calf and thigh, compared to running which activates muscle groups in the entire body. Running may have had a different effect on appetite regulation since more muscles are recruited and a greater amount of biomarkers that affect appetite could have been secreted. However, the capacity to conduct weight-bearing exercise, such as walking, is reduced in OB compared to NW (Maatman et al., 2016) and may have compromised experiment 1 (Chapter 4). In the scope of this study and to ensure comparability between NW and OW/OB in experiment 1 (Chapter 4) and NW experiment 2 (Chapter 5) we chose a non-weight-bearing exercise on a recumbent bicycle.

Females were not included in the study due to logistical reasons. Currently, there is only limited evidence that appetite differs in males and females with EX (Thackray, Deighton, King, & Stensel, 2016). Conducting appetite research in children and adolescents is difficult as hormonal changes can cause appetite and FI to vary, especially in females. If females reach a pubertal age, menstrual cycle phase-dependent changes in appetite start to manifest mediated by pituitary hormones such as follicle-stimulating hormone, progesterone, and estrogen, luteinizing hormone and gonadotropin-releasing hormone (Hambridge et al., 2013). Females also display different metabolic profiles in regards to inflammation (Cartier et al., 2009; Mathad et al., 2016) as well as appetite hormones (Bedard et al., 2015; Williams et al., 2016). However, exploring sex differences in regard to appetite regulation with HIEX and GLU/PRO preloads was out of the scope of this project and for logistic reasons, we were not able to assess sex hormones before the start of the study or include enough female participants to offset the cyclic variability in FI. In addition, and for similar reasons, we did not account for pubertal stage in these experiments. The pubertal
stage can be determined through tanner staging and other biomarker measurements. Both would have significantly increased logistic and financial barriers associated with this research.

A significant barrier to appetite and FI studies is the differentiation between appetite caused by metabolic needs or hedonic mechanisms. Appetite and FI in humans are driven by both. It is difficult to determine the effect of hedonic mechanisms and the effect of EX and macronutrients on FI. Food can send a strong signal, and it is not currently known how physiologic mechanisms such as appetite hormones, inflammatory biomarkers, and hedonic mechanisms interact to control FI, especially with EX. Furthermore, appetite responses to EX can alter drastically from individual to individual. One study showed that individual responses in appetite to EX vary considerably, but it needs to be determined how much hedonic and physiological mechanisms interact with each other and affect subsequent appetite and FI (Champagne et al., 2013).

One further limitation of this study was the exclusion of OW/OB in experiment 2 and 3 (Chapter 5 and 6). Our first experiment (Chapter 4) was conceptualized as the pilot study, preparing the setup and design of the following two studies. We did not see an effect of BW in our first experiment; therefore, we did not pursue the effect of BW in our later studies. Although there may have been additional results and insights into OW/OB and NW children and adolescents, we would have needed to include much larger sample size, increasing the logistic and financial burden of this project significantly. We also assessed FI as a measurement of kcal per kg Body-Weight rather than an absolute measure to account for differences in Body-Weight due to age and WS in our participants. The accurate measurement of FI and the ability to compare various studies with each other is often difficult. A limitation of measuring FI on an absolute basis
is that FI in kcal will differ significantly in individuals displaying low or high Body-Weights due to their varying energy requirements. This will, in turn, make it problematic to compare the change of kcal in response to treatment in individuals who consume more food because the magnitude of the effect can significantly differ. This can be mediated by adjusting FI to kcal/kg, making a comparison between low and high eaters more feasible. However, a comparison adjusted to kcal per kg Body-Weight does not account for adipose tissue as metabolically “inactive”. One more potential strategy to assess FI is the adjustment for fat mass. Bioelectrical impedance analysis (BIA) is used as a standard and inexpensive procedure to assess fat mass. However, it is dependent on several factors such as hydration status, race, and the equipment and equations being used (Ireton-Jones, 2005). In addition, equations for the measurement of fat mass in OB adolescents were not validated at the time this research was conducted and only became available recently (Steinberg et al., 2019). To be able to draw comparisons to other studies and eliminate potential errors associated with the BIA measurement we decided to measure FI on a kcal per KG basis.
7.3. **Significance and Implications:**

Through this research and literature review, we were able to expand our previous model of FI regulation ([Figure 11. 1 – Appendix](#)). FI regulation is a precise process that involves the amalgamation of complex homeostatic mechanisms in the CNS triggered by peripheral signals (G. H. Anderson, Aziz, & Abou Samra, 2006; G. H. Anderson et al., 2016; Chambers, Sandoval, & Seeley, 2013). These signals contain sensory properties of foods, mechanical and chemical receptors in the GI tract, gut hormones, and circulating metabolites (Cummings & Overduin, 2007; Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). The hypothalamus, brainstem, and cortex integrate these signals, and decode them into information, controlling meal size and duration; interval to the next meal; the amount of food eaten throughout the day or over several days, weeks, and months; and possibly the composition of food, as well as the intake of total energy. This research has emphasized the importance of physical activity and inflammatory biomarkers to our previous understanding of FI regulation as shown by the red additions in **Figure 11. 1**.

Furthermore, these three experiments advance our understanding of inflammation and the relationship appetite regulation in children, a thus far neglected area of research. This research offers the groundwork for providing practical advice aimed at children to prevent overeating and weight gain. To our knowledge, these are the first experiments advancing the basic understanding of appetite regulation mediated through inflammation in children. This research has implications for the development of recommendations for EX and nutrition programs for children, helping to time EX routines and meal schedules to achieve a caloric deficit.
A greater understanding of the interplay between EX, inflammation and appetite may help to shape school policies, and recess and lunch timing.
Chapter 8
GENERAL SUMMARY AND CONCLUSIONS

In the experiments with HIEX (Chapter 4 and 5), IL-6 was the strongest predictor of appetite, closely followed by active ghrelin. However, with GLU and PRO ingestion, (Chapter 6), an acute response in IL-6 was not detected, and GLP-1 and insulin were more strongly associated with appetite and FI. However, the inverse association of IL-6 with FI independent of a treatment effect also suggest a role for IL-6 in appetite control. Thus, this research leads to the suggestion that the role of IL-6 in appetite control maybe by different mechanism after HIEX than in the regulation of food-induced regulation. Therefore, HIEX and the ingestion of food should be seen as separate appetite and FI regulatory processes that need to be investigated under different parameters.
Chapter 9
FUTURE DIRECTIONS

Several ideas on how to advance the conclusions of the current experiments are proposed in the following. Currently, the effect around the exercise-induced suppression is not well defined; this includes the exercise mode, intensity, duration, time to next meal as well as the population studied. Various intensity and duration protocols could be used to conduct the EX sessions. Our protocol engaged children and adolescents in three ten-minute bouts at either 70% VO$_2$max or 75% VO$_2$max. Currently, it is unknown which exercise intensity or duration is needed to induce suppression of appetite as well as FI in children and adolescents. For example, one experiment showed a decrease in FI compared to rest and lower EX intensity, utilized an exercise protocol at a very high intensity, exercising for 170% VO$_2$peak for 15 seconds and alternating with 60 seconds at 32% VO$_2$peak for 30 min (Sim et al., 2014). Exercise duration may also play a role, one study found no overall differences in overall FI but saw differences in macronutrient intake at an ad libitum meal after exercising either 20 min or 40 min at the V$_{LT}$, indicating an effect no on overall food intake but macronutrient preference (Masurier et al., 2018). Time to next meal may also play a role, in our second experiment (Chapter 5) we saw a suppression of appetite immediately after HIEX which already recovered to resting values within 15 min. Therefore, decreasing the time between HIEX and the initiation of the lunch meal may be crucial to observe a suppression of FI. Future studies should test various intensities, durations, and time to next meal protocols, by increasing the intensity/duration simultaneously while decreasing the time to next meal. This way it may be possible to gradually create a more significant stimulus affecting FI regulation and identify the threshold where FI is consistently
suppressed. This could then be repeated using various EX modalities, such as swimming, running, cycling or resistance exercise. EX-induced anorexia needs to be explored further in groups of people with different metabolic profiles. For example, NW and OB have shown different appetite hormone- (Lean & Malkova, 2016), and inflammatory-profile. Males and females also have shown different appetite responses to HIEX (Hallam, Boswell, DeVito, & Kober, 2016). Some research suggests that there is a high inter and intra-individual variability in appetite response to HIEX, which may be mediated by different metabolic profiles of different individuals (Stupka et al., 2000). Studies should investigate each group against a healthy control group, using a sufficient sample size.

Several steps could be taken to isolate the effect of specific biomarkers affecting appetite. Vagal afferents innervating the GI tract, provide rapid and accurate information regarding the number of digestible macronutrients in the alimentary tract. At the same time vagal efferent, hormonal mechanisms, and the sympathetic nervous system are determining the rate of nutrient absorption, partitioning, storage, and mobilization. Vagal afferents are also present in gastric mucosa, where they can detect locally released hormones such as ghrelin and leptin. One study showed that ghrelin’s appetite-stimulating effects were eliminated in rats with sub-diaphragmatic vagotomy and rats with capsaicin-induced vagal deafferentation (Date et al., 2002). A study using vagotomized rats and their healthy counterparts as control could further explore the effect of appetite hormones and inflammatory biomarkers on appetite and FI regulation with HIEX. By vagotomizing rats the appetite-inducing effects of ghrelin could be suppressed, and the effects of IL-6 on FI could be studied, in comparison to healthy rats. Similarly, in humans, who just underwent bariatric surgery, and their healthy counterparts, could take part
in similar study design, since the changes in appetite and FI behaviour have been, at least in part, related to changes in GI hormones acting on vagal afferents (Berthoud, 2008).

Patients with cachexia could also serve as a model to explore the effects of HIEX on appetite and FI regulation. Cachexia is defined as muscle atrophy, weakness, loss of weight, fatigue, and a significant loss of appetite. Cachexia is often seen in cancer, in patients with congestive heart failure, and patients with chronic obstructive pulmonary disease, increasing disease progression and mortality risk (Ebner et al., 2013). For example, cachexia accounts for approximately 20% to 40% of all cancer-related deaths and is directly linked to mortality and morbidity (Tisdale, 2009). Further illnesses with cachexia can include cystic fibrosis, motor neuron disease, chronic kidney disease, HIV/AIDS, multiple sclerosis, Parkinson's disease, dementia, and other progressive illnesses (Payne, Wiffen, & Martin, 2017). The etiology of cachexia has not been fully understood; however, chronic systemic inflammation is present in the majority of patients, and especially IL-6, TNF-α and CRP plays a pivotal role in cancer cachexia (Argiles, Busquets, & Lopez-Soriano, 2011; de Matos-Neto et al., 2015), potentially mediating the loss of appetite. Therapeutic approaches to treating cachexia often target inflammation via anti-inflammatory agents. They may improve weight in cancer patients with cachexia. In addition, there is some evidence on the effect on quality of life, physical performance, and inflammatory biomarkers, but current evidence is not robust enough to draw conclusions (Solheim et al., 2013). Recently, EX has been proposed as another potential therapeutic approach manage cachexia via the modulation of insulin sensitivity, muscle metabolism, and inflammation (Maddocks, Jones, & Wilcock, 2013; Maddocks, Murton, & Wilcock, 2012). EX has already been shown to be beneficial
in patients with cancer (Heywood, McCarthy, & Skinner, 2018); however, evidence of the effect of EX on cachexia is lacking and insufficient to determine the safety and effectiveness.

Further studies should explore the effect of acute and long-term EX in cachectic patients on appetite and inflammatory biomarkers and the subsequent effect on appetite regulation and FI. One approach could be to investigate appetite and FI control in patients showing early onset of cancer cachexia in comparison to cancer patients without cachexia. Parallel studies in animal models could help to gain a more mechanistic understanding of the central regulation of appetite in cachectic animals prior and post EX regimen.

Inflammation has also been shown to induce differences in post-prandial fatigue. Previous research linked the IL-1 family to postprandial sensations of fatigue, which was more pronounced in OB when compared to lean individuals (Lehrskov et al., 2018). Understanding inflammatory induced post-prandial fatigue could have further implications on meal timing and exercise performance. Studies could focus on athletes and meal timing, blocking IL-1 and investigating the effect on EX performance. A further application could be within rheumatic arthritis, a condition that is characterized by severe fatigue (Alten et al., 2011). Blocking IL-1 might help to reduce fatigue in these patients, but evidence is still lacking.

Further, inflammatory and appetite biomarkers should be explored. In this Ph.D. experiment, we focused on the most prominent biomarkers that have been explored with HIEX and macronutrient consumption. However, other inflammatory biomarkers and gut peptides have also been implied in FI and appetite control. IL-1β, for example, has been shown to be a potent appetite suppressant (Plata-Salaman, 1995), similar to IL-10 and INF-γ; however, in this
study, we only focused on inflammatory biomarkers that were linked to HIEX (Kasapis & Thompson, 2005).
Chapter 10
References


type 2 diabetic subjects. *Endocrinology, 139*(12), 4793-4800. doi: 10.1210/endo.139.12.6368


Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? 


Hunschede, S., Kubant, R., Akilen, R., Thomas, S., & Anderson, G. H. (2017). Decreased Appetite after High-Intensity Exercise Correlates with Increased Plasma Interleukin-6 in Normal-


Immune Response to Dietary Excess and Mediates Obesity Susceptibility. *Cell Metab*, 26(1), 185-197 e183. doi: 10.1016/j.cmet.2017.05.015


Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab, 86*(12), 5992. doi: 10.1210/jcem.86.12.8111

Ghrelin causes hyperphagia and obesity in rats. *Diabetes, 50*(11), 2540-2547.

Acute Lesioning and Rapid Repair of Hypothalamic Neurons outside the Blood-Brain Barrier. *Cell Rep, 19*(11), 2257-2271. doi: 10.1016/j.celrep.2017.05.060

Chapter 11
Appendix

Food Intake Regulation

<table>
<thead>
<tr>
<th>Input</th>
<th>CNS</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long-term regulation</strong></td>
<td><strong>Long-term regulation</strong></td>
<td></td>
</tr>
<tr>
<td>Adipose Tissue</td>
<td>Cortex</td>
<td>Size of energy stores</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hypothalamus</td>
<td>Seasonal Food Intake</td>
</tr>
<tr>
<td>Leptin</td>
<td>Brainstem</td>
<td>Tonic Inflammatory biomarkers</td>
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<tr>
<td>Physical Activity</td>
<td></td>
<td></td>
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<tr>
<td><strong>Short-term regulation</strong></td>
<td><strong>Short-term regulation</strong></td>
<td></td>
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<tr>
<td>Sensory signals</td>
<td></td>
<td>Satiety</td>
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<tr>
<td>GI Signals</td>
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<td>Satiation</td>
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<tr>
<td>Circulating signals</td>
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<td>Meal Duration</td>
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<td>Episodic Inflammatory biomarkers</td>
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<td>Intermeal Interval</td>
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<td></td>
<td>Food Preference</td>
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</tbody>
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**Figure 11. 1:** Physiology of FI regulation with the addition of the effect of inflammatory biomarkers and PA. Adapted with permission from reference (G. H. Anderson, Aziz, & Abou Samra, 2006).