2016

The Effects of Exercise Intensity on Acylated Ghrelin, Active Glucagon-like Peptide-1, and Appetite: Examining The Potential Involvement of Interleukin-6

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THE EFFECTS OF EXERCISE INTENSITY ON ACYLATED GHRELIN, ACTIVE GLUCAGON-LIKE PEPTIDE-1, AND APPETITE: EXAMINING THE POTENTIAL INVOLVEMENT OF INTERLEUKIN-6

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A thesis submitted to the Faculty of Graduate and Post-doctoral Studies in partial fulfillment of the requirements for the Master of Science degree

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ABSTRACT

Interleukin-6 (IL-6) stimulates the release of appetite-regulating hormones in animals and associates with decreased energy intake in humans. Thus, IL-6 may contribute to the intensity-dependent effects of exercise on appetite-related parameters. The purpose of this study was to examine the effects of exercise intensity on IL-6, appetite-regulating hormones, and appetite perceptions. Eight active young males completed four sessions: 1) Moderate-intensity continuous training (MICT; 30 min running, 65% VO_{2max}); 2) High-intensity continuous training (HICT; 30 min running, 85% VO_{2max}); 3) Sprint interval training (SIT; 4 x 30 sec "all-out" running bouts separated by 4 min recovery); 4) Control (CTRL; no exercise). Blood samples were obtained immediately pre- and post-exercise, as well as 30- and 90-min post-exercise for the measurement of acylated ghrelin, active glucagon-like peptide-1 (GLP-1), and IL-6. Appetite perceptions were assessed at the same time-points using a visual analog scale. Energy intake was recorded for a 3-day period beginning on the day before each session. Acylated ghrelin and appetite were suppressed after HICT ($P<0.005$) and SIT ($P<0.002$), though more so after SIT compared to MICT ($P<0.042$). Active GLP-1 concentrations increased immediately after MICT ($P<0.001$) and 30 min after HICT ($P<0.001$) and SIT ($P=0.005$). Intensity-dependent increases in IL-6 coincided with decreases in acylated ghrelin and correlated negatively with appetite after HICT. Though not correlated, simultaneous increases in GLP-1 and IL-6 were observed 30 min after HICT and SIT. Free-living energy intake was reduced on the day after HICT compared to both MICT ($P=0.028$) and CTRL ($P=0.020$). These findings support an intensity-dependent paradigm for appetite-regulation that is strongly associated with changes in acylated ghrelin and may be mediated by IL-6.

ACKNOWLEDGEMENTS

To my supervisor, Tom:
I wouldn't be here today if you hadn't given me this opportunity, and for that I will be forever grateful. Thanks for your guidance, support, and most importantly your friendship over these last few years. I could not have asked for a better supervisor and hope to make you proud as I progress through my academic career. Looking forward to many more years of collaboration, conferences, and everything in between.

To my committee members, Dr. Kalmar and Dr. Gurd:
It has been a true privilege to have your invaluable scientific insight for my project. Thank you for contributing your time and expertise.

To my lab mates, Logan and Greg:
EMRL would not have been the same without you boys.

To my loving parents, Sadia and Umar:
I owe everything I have achieved to the two of you. Thank you for always supporting me in every aspect of my life.

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CHAPTER 2 - MANUSCRIPT

LIST OF ABBREVIATIONS

AEBSF – 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
AgRP – agouti-related peptide
ANOVA – analysis of variance
ARC – arcuate nucleus
AUC – area under the curve
BMI – body mass index
CART – cocaine- and amphetamine-related transcript
CCK – cholecystokinin
CNS – central nervous system
CTRL – control
DPP-IV – dipeptidyl peptidase-4
EDTA – ethylenediaminetetraacetic acid
FFA – free fatty acid
GLP-1 – glucagon-like peptide-1
GOAT – ghrelin O-acyl transferase
HICT – high-intensity continuous training
HIIT – high-intensity interval training
HR – heart rate
HR_{max} – maximum heart rate
IL-6 – interleukin-6
MICT – moderate-intensity continuous training
NPY – neuropeptide Y
PAR-Q – physical activity readiness questionnaire
POMC – pro-opiomelanocortin
PP – pancreatic polypeptide
PYY – peptide tyrosine tyrosine
RER – respiratory exchange ratio
SIT – sprint interval training
VAS – visual analog scale
VCO_2 – carbon dioxide production
VO_2 – oxygen consumption
VO_{2max} – maximal oxygen consumption

CHAPTER 1:

LITERATURE REVIEW

1. Introduction

The prevalence of overweight and obesity has reached epidemic levels worldwide with over 1.9 billion adults (39%) who were overweight and 600 million (13%) who were obese in 2014 (1). Physical inactivity and unhealthy dietary habits are likely the main culprits, given the readily available sources of energy-dense foods and increasingly sedentary lifestyles. The increased body mass index (BMI) in both overweight (BMI $\geq$25 kg/m^2) and obese (BMI $\geq$30 kg/m^2) individuals is accompanied by an elevated risk of various chronic diseases (i.e. diabetes, hypertension, coronary heart disease, stroke, some cancers) that greatly strains healthcare resources (2). Clearly, there is an urgent need for cost-effective interventions that successfully promote weight loss leading to a healthier body composition.

Energy balance

Energy balance involves the interplay between energy intake and energy expenditure, and is the key concept underlying body weight regulation (3). Weight gain (i.e. increase in fat mass) that eventually leads to all out obesity results when energy intake consistently exceeds energy expenditure during prolonged and repeated periods of energy excess (4). On the contrary, a maintained energy deficit is required for effective weight loss (i.e. decrease in fat mass) to occur, and is typically achieved by increasing energy expenditure, decreasing energy intake, or a combination of both. Given that food intake and exercise energy expenditure are both highly modifiable lifestyle factors that regulate body weight, they provide key targets for obesity prevention and reduction (5).

While seemingly unsophisticated, the physiological (6), psychological (7) and environmental (8) inputs that influence energy balance have made it difficult to manipulate its components effectively. Although exercise is often utilized to improve body composition

through increases in energy expenditure, weight loss following traditional aerobic exercise interventions (12 wk – 1 y) is often inadequate (<2 kg), even when performed in amounts (>150 min/wk at a moderate intensity) prescribed by the American College of Sports Medicine (9,10) . This may be due to compensatory increases in appetite that stimulate energy intake and nullify the exercise energy expenditure, thus preventing the energy deficit required for weight loss (11,12). While both components of energy balance must be targeted for combatting the obesity epidemic, increases in body weight over recent decades appear to be driven more strongly by drastic elevations in energy intake rather than reduced energy expenditure (13). Clearly the design and implementation of exercise interventions should consider their subsequent effects on energy intake in addition to their effects on energy expenditure. Therefore, exercise protocols that sufficiently increase energy expenditure without promoting subsequent increases in appetite and/or energy intake are highly desirable.

High-intensity exercise

The impact of exercise intensity on overall health is readily apparent in current physical activity guidelines that allow for prolonged bouts of moderate-intensity activity (150 min/wk) to be substituted with vigorous exercise of lower duration (75 min/wk) (14). In fact, intense exercise is associated with greater all-cause mortality reduction than moderate-intensity activity of equal volume and accumulating evidence supports proportionally greater cardiovascular benefits with increasing exercise intensity (15,16). The reduced time commitment associated with vigorous activity is also appealing to individuals who fail to achieve adequate exercise due to a perceived lack of time (17). Therefore, high-intensity exercise protocols have become increasingly popular in the health and fitness field, typically in the form of low-volume interval training (18). While these protocols vary in terms of the exact intensity, duration, and number of

intervals, the main goal is simply to perform a greater amount of intense exercise per session. This is facilitated by the inclusion of brief rest periods that allow the repeated completion of intense work bouts that would not otherwise be sustainable during prolonged continuous exercise, resulting in a stronger adaptive stimulus that leads to greater health benefits (15,19).

High-intensity interval training (HIIT) involves brief bouts of near maximal (80-100% HR_{max}) activity followed by short recovery periods and promotes similar physiological adaptations to moderate-intensity (50-75% VO_{2max}) continuous training (MICT) despite significantly less exercise time involved (18). More importantly for energy balance, cycling-based HIIT has been shown to induce fat loss that is comparable to or greater than MICT (20-24). Similar benefits are achieved with a more intense form of intermittent exercise known as sprint interval training (SIT) that involves supramaximal efforts (>100% VO_{2max}), typically structured as four to six 30 second "all-out" efforts separated by 4 min recovery periods (18). This model of running-based SIT has been shown to induce comparable (or potentially greater) fat loss (1.2-1.7 kg) to MICT (0.8 kg) over 6 weeks despite a fraction of exercise duration (2-3 min of SIT vs. 30-60 min of MICT per session) and lower overall time commitment (25,26). Due to the brief nature of HIIT and SIT, the observed improvements in body composition have been attributed to protracted increases in post-exercise metabolism (27-30) that result in 24 h energy expenditure that is remarkably similar to MICT (29,31). Additionally, these protocols have been shown to promote an acute substrate shift that favors fat utilization after exercise (27,28,32,33) and chronically up-regulate enzymes and proteins involved in fat oxidation (21,34-38) and transport (39).

Despite the acute increases in energy expenditure and fat utilization that are reported following HIIT/SIT, some studies have suggested that the magnitude of these effects is likely

insufficient for explaining the fat loss observed with these protocols (40-42). As such, improvements in body composition may also be attributable to changes in other aspects of energy balance, potentially through alterations in appetite and/or energy intake (43,44). Despite the intense nature of HIIT/SIT, these protocols do not appear to promote compensatory increases in feeding (27,45) and may even suppress appetite (42,46,47) and subsequent energy intake (45,48,49) more so than MICT. Interestingly, these appetite suppressive effects are also observed with more strenuous (≥70% VO_{2max}) versions of MICT, highlighting exercise intensity as a key stimulus (50-52). These observations are consistent with the intensity-dependent nature of several mechanisms that have been proposed to mediate changes in appetite and/or energy intake following exercise (53). However, apart from a limited number of studies comparing HIIT/SIT to MICT, there is currently a lack of evidence on the direct effects of exercise intensity on the regulation of energy intake. Nevertheless, the health benefits of high-intensity exercise protocols (both continuous and intermittent) as well as their potential to influence both energy expenditure and energy intake makes them an attractive model for understanding energy balance. In order to elucidate the effects of exercise intensity on energy intake, a brief overview of key physiological mechanisms and their responsiveness to exercise is first necessary and as such will be the focus of the subsequent section.

2. Physiological regulation of energy intake

Overview

The physiological mechanisms that govern energy intake involve the interaction between the key brain regions involved in energy homeostasis and peripheral organs/tissues that secrete "orexigenic" (i.e. appetite-stimulating) or "anorexigenic" (i.e. appetite-inhibiting) hormones

(Figure 1). Though several hormones have been implicated in the control of energy intake, acute appetite is primarily regulated by episodic signals released from the gastrointestinal tract that provide information on the short-term energy status of the body (6). Consequently, these hormones influence feeding behavior by altering perceptions of hunger and satiety, which are associated with meal initiation and termination, respectively (54). Depending on the energy status of the body, these hormones act in a homeostatic fashion to increase or decrease appetite in order to restore energy balance through altered energy intake (55). Periods of energy surplus or deficit (i.e. between meals) promote fluctuations in circulating hormone concentrations largely through mechano- (i.e. intraluminal distention) and chemo- (i.e. presence of nutrients) sensory mechanisms in the gastrointestinal tract (54). Hereafter, these signals converge directly (i.e. blood brain barrier) and indirectly (i.e. vagal input, CNS receptor activation) on the brain and are integrated primarily in the arcuate nucleus (ARC) of the hypothalamus (56). Within the ARC, there exist distinct neuronal populations that play a critical role in appetite control by modulating neuropeptide release in response to peripheral signals that reflect energy status (57). Specifically, orexigenic neurons secreting neuropeptide Y (NPY) and agouti-related peptide (AgRP) act to stimulate appetite, while anorexigenic neurons that produce pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART) promote decreases in appetite (57). The neuronal circuits of the ARC also communicate with other hypothalamic regions that influence food intake, such as the paraventricular, dorsomedial, and ventromedial nuclei (58). Other important brain regions include the caudal brainstem, which facilitates communication between the hypothalamus and the periphery, and the corticolimbic system, which integrates non-homeostatic factors such as hedonism, environmental cues, and palatability (6). While these factors are certainly important determinants of energy intake under free-living conditions, it

becomes increasingly difficult to quantify non-homeostatic variables in a laboratory setting (59). Therefore, the subsequent discussion of appetite regulation will be limited to homeostatic mechanisms focusing on key gastrointestinal hormones that influence hunger and satiety.

Gastrointestinal hormones

Ghrelin – Ghrelin is a 28 amino acid peptide hormone released predominantly from endocrine cells in the stomach and the only peripheral signal known to stimulate appetite (60). Plasma concentrations are elevated in the fasted state, peaking immediately before feeding and declining post-prandially in proportion to caloric load, which supports its role an episodic hunger signal involved in meal initiation (61,62). Thought the exact mechanisms regulating ghrelin release are unclear, carbohydrate intake appears to be a strong suppressive stimulus potentially due to inhibitory effects of glucose and/or insulin action on this hormone (63,64). Administration of exogenous ghrelin has been shown to potently stimulate food intake in both rodents (65) and humans (66). Endogenously produced ghrelin requires the addition of an eight-carbon fatty acid (octanoyl) by the enzyme ghrelin O-acyl transferase (GOAT) in order exert its orexigenic effects (67). Hereafter, the acylated (active) form crosses the blood-brain barrier and exerts its effects in the hypothalamus where it stimulates the NPY/AgRP neurons that increase appetite (68,69). Ghrelin levels inversely correlate with body weight and tend to rise after diet-induced weight loss (70). Circulating concentrations tend to be lower in obese individuals and less responsive to food intake compared with those that are lean, which suggests that excessive weight gain may impair ghrelin's regulatory effects on appetite and further contribute to the pathogenesis of obesity (71).

Peptide YY (PYY) – PYY is an anorexigenic hormone secreted predominantly from the endocrine L cells located in the distal small intestine and belongs to the same family of peptides

as the orexigenic neurotransmitter NPY (72). The initial product PYY_{1-36} is rapidly proteolyzed by the enzyme dipeptidyl peptidase-4 (DPP-IV) to PYY_{3-36}, which represents the major circulating form of this peptide both in the fed and fasted state (51,73). Though both forms are considered biologically active, only PYY_{3-36} exhibits a high affinity for the Y2 presynaptic inhibitory receptors of NPY neurons to which it binds, while also stimulating the anorexigenic POMC neurons to suppress appetite (72,74). Peripheral PYY_{3-36} infusion has been shown to potently inhibit food intake in animals as well as lean and obese humans (74-76). Postprandial PYY concentrations rise in proportion to caloric intake and are highest immediately after a meal, which supports its role as an episodic satiety signal involved in meal size and termination (76,77). Though protein rich meals appear to elicit the greatest postprandial PYY response, fat content has also been suggested to play a role (6,72). The satiating effects of PYY are also mediated by its ability to delay gastric emptying via the ileal break reflex that inhibits gastric motor activity in response to intestinal nutrient stimulation (78). Although obese individuals are not resistant to the anorexigenic effects of PYY_{3-36}, they tend to exhibit lower fasting and postprandial levels compared to those who are lean, suggesting that impairments in meal-induced satiation may play an important role in the development of obesity (79).

Glucagon-like Peptide-1 (GLP-1) – GLP-1 is the incretin product of posttranslational modifications to the preproglucagon gene that is released from the intestinal L cells, acting to stimulate insulin release and inhibit glucagon secretion (54). As such, carbohydrate ingestion appears to be the strongest stimulus for GLP-1 release though fat intake also plays a role (54). Apart from its incretin effects, both central and peripheral GLP-1 administration has been shown to potently inhibit food intake and appetite in both humans (lean and obese) and animals (80-83). The anorexigenic potential of GLP-1 has also been linked its ability to stimulate the ileal brake

reflex and suppress gastric acid secretion (81). GLP-1 is co-released with PYY due to their common secretory origin, and the two hormones appear to have synergistic effects on appetite suppression (84). Two equipotent forms of this hormone ($GLP\text{-}1_{7\text{-}36}$ and $GLP\text{-}1_{7\text{-}37}$) are present in circulation, with $GLP\text{-}1_{7\text{-}36}$ being the more abundant form, and both are rapidly degraded to the inactive truncated form $GLP\text{-}1_{9\text{-}36}$ by DPP-IV, which also metabolizes $PYY_{1\text{-}36}$ to $PYY_{3\text{-}36}$ (85). Receptors for active GLP-1 can be found in the brainstem, hypothalamus, and throughout the periphery (86). Consistent with its ability to cross the blood-brain barrier, GLP-1 induced anorexia has been shown to involve central pathways targeting brainstem and hypothalamic (i.e. ARC) neurons, though vagotomy appears to abolish some of these effects suggesting that signaling through vagal afferents is also important (54,87). Similar to PYY, the anorexigenic effects of peripherally administered GLP-1 are preserved in obesity, though the post-prandial rise in GLP-1 appears to be delayed and potentially of lesser magnitude than in lean individuals (81).

Though several other hormones are involved in energy homeostasis, they were excluded from this review, which was primarily focused on hormones demonstrated to respond to acute energetic perturbations (i.e. exercise) and influence energy intake over a short time period (i.e. meal to meal). For example leptin, the extensively studied product of the ob gene, exhibits anorexigenic properties acting to decrease energy intake though changes in this hormone do not seem to occur after acute exercise and instead reflect long-term changes in energy stores (i.e. training-induced changes in fat mass) (43). Other gastrointestinal signals such as cholecystokinin (CCK), amylin, and pancreatic polypeptide (PP) have also been shown to exert anorexigenic properties though changes in these peptides are either not well documented in response to acute exercise (i.e. CCK, amylin) or have shown to be inconsistent (i.e. PP) (53).

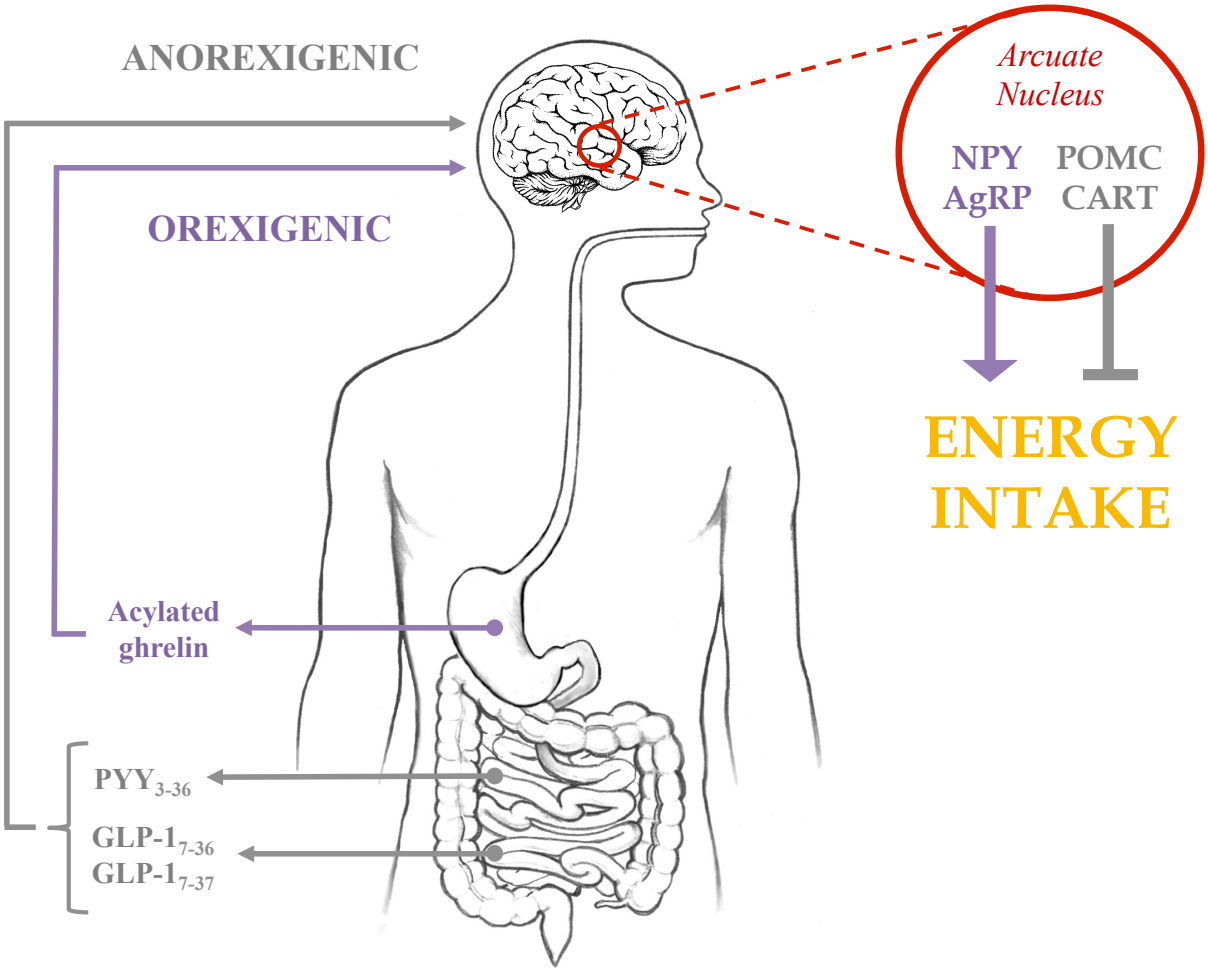

Figure 1. Schematic overview of key gastrointestinal hormones and neuropeptides involved in the physiological regulation of energy intake. AgRP: agouti-related peptide; CART: cocaine- and amphetamine-related transcript; GLP-1: glucagon-like peptide-1; NPY: neuropeptide Y; POMC: pro-opiomelanocortin; PYY: peptide YY.

3. Effects of acute exercise

Moderate-intensity continuous training (MICT)

The majority of research examining the effects of aerobic exercise on acute (0-9 h post-exercise) appetite regulation has involved moderate-intensity continuous training (MICT; 50-75% VO_{2max}). Though several studies have examined changes in total ghrelin following MICT protocols involving 30-90 min of cycling, running, or rowing, the majority have failed to show any significant exercise effects on circulating concentrations (50,53,88-96). Two of these studies also measured the active form of this peptide, acylated ghrelin, and indeed reported reduced post-exercise concentrations despite no changes in total ghrelin (93,95). As such, total ghrelin appears to be less important for appetite regulation than acylated ghrelin and not indicative of changes in the active form of this hormone (53,97). The suppressive effects of MICT on acylated ghrelin concentrations have been confirmed by numerous other studies showing reductions of 14-60% versus pre-exercise or resting levels, with the greatest effects observed after more prolonged (60-90 min) protocols or those performed at the higher (≥70% VO_{2max}) end of the MICT intensity range (47,51,95,98-108). Several of these studies have also reported concomitant reductions in hunger, which supports the orexigenic nature of this peptide (51,92,99,100,103,105). However, others have failed to show reductions in acylated ghrelin following MICT, suggesting that a more intense stimulus may be needed to elicit consistent effects (49,92,102,109-111).

In terms of anorexigenic gut peptides, studies examining the effects of MICT (30-90 min) on total PYY (PYY_{1-36} and PYY_{3-36}) have demonstrated post-exercise elevations ranging from 8-172% in comparison with baseline or resting values (47,51,88,96,100,109), while others have shown no change (49,93,98,101,112). These increases appear to be much more robust (11-

2200%) and consistent when studies have exclusively measured PYY_{3-36}, which has been implicated as the more potent regulator of appetite (46,92,104,111,113-115). Furthermore, a direct comparison between two MICT protocols involving 50% and 75% VO_{2max} cycling for 30 min revealed intensity-dependent effects for this peptide, supporting a beneficial role of exercise intensity (115). Though much less research has focused on GLP-1, cycling and running based MICT (30-60 min) has been shown to increase both total GLP-1 as well as the active forms ($GLP\text{-}1_{7-36}$, $GLP\text{-}1_{7-37}$) of this peptide in the acute post-exercise period (92,96,115-117). Unlike PYY_{3-36}, increasing the intensity of MICT from 50% to 75% does not seem to influence active GLP-1 concentrations (115). True to the satiating nature of these peptides, several of the aforementioned studies have also reported reductions in appetite and/or energy intake in association with increased PYY and/or GLP-1 concentrations (51,92,96,100,115).

Collectively it appears that MICT induces acute post-exercise decreases in the hunger hormone acylated ghrelin while promoting increases in the satiating gut peptides PYY and GLP-1. These changes promote an anorexigenic environment that favors decreases in energy intake and, given the episodic nature of these signals, allow for improved short-term energy regulation following exercise. Nevertheless, the lack of effect in some studies as well as the potentially greater effects observed with more intense versions of MICT suggests that higher intensity exercise may be a more potent appetite-regulatory stimulus.

High-intensity/sprint interval training (HIIT/SIT)

There is currently a lack of information on the effects of high-intensity continuous training (HICT; >75% VO_{2max}) on appetite related parameters. As such, examination of the effects of exercise intensity on appetite regulation has been limited to intermittent exercise protocols. Deighton and colleagues (46) demonstrated that HIIT (10 x 4 min cycling intervals at

85% VO_{2max}, 2 min rest) resulted in a greater suppression of appetite as well as greater elevations in PYY_{3-36} concentrations compared to MICT (60 min cycling at 60% VO_{2max}) in healthy males. Furthermore, these changes were also accompanied by reductions in relative energy intake (energy intake minus expenditure during exercise) supporting the ability of HIIT to induce an acute energy deficit (46). Data from the same group has also shown that the more intense SIT protocol (30 s "all-out" cycling x 6 bouts, 4 min recovery) resulted in a greater suppression of appetite and acylated ghrelin than MICT (60 min cycling at 65% VO_{2max}) (47). Although one study reported an increase in overall hunger following SIT, total PYY briefly increased post-exercise and energy intake remained unaffected despite significant increases in energy expenditure for 34-h post-exercise (27). In support of the hormonal data, an examination of energy intake responses following exercise reported a lower compensatory response following HIIT as compared with MICT in terms of hunger, desire to eat, and fat intake (48). Similar findings have been reported in a study that compared energy intake in overweight men following MICT (30 min cycling at 60% VO_{2peak}), HIIT (1 min cycling at 100% VO_{2peak} followed by 4 min at 50% VO_{2peak} x 6 bouts), and SIT (15 s cycling at 170% VO_{2peak} followed by 1 min at 32% VO_{2peak} x 15 bouts) (49). SIT resulted in the greatest suppression of acylated ghrelin as well as a lower energy intake than MICT that persisted for 24 h (49). Suppression of acylated ghrelin and increased postprandial release of GLP-1 has also been reported in obese individuals following an acute bout of HIIT with no compensatory increases in appetite (116). Overall, emerging evidence suggests that acute HIIT and SIT modulate energy regulating hormones to produce an anorexigenic effect that has the potential to reduce energy intake. More importantly, the intense nature of such protocols does not stimulate compensatory increases in appetite and/or energy intake. Given the limited number of studies examining appetite regulation following HIIT/SIT,

additional research is required to fully elucidate the importance of exercise intensity. Furthermore, the lack of information regarding the effects of HICT has made it difficult to establish a clear dose-response relationship between exercise intensity and appetite-regulation.

4. Interleukin-6

The mechanisms underlying changes in appetite-regulating hormones following acute exercise are poorly understood though we have recently highlighted several mechanisms that support a beneficial role for high-intensity exercise. These include changes in gastrointestinal blood flow and motility, sympathetic nervous system activity, muscle metabolite turnover, plasma free fatty acids, and blood glucose/insulin concentrations (53). A particularly attractive and largely uninvestigated mechanism involves the contraction mediated cytokine interleukin-6 (IL-6).

Though initially believed to be a component of the acute phase inflammatory response indicative of muscle damage, it is now well established that IL-6 release during exercise originates from working skeletal muscle rather than immune cells (118). Furthermore, muscle-derived IL-6 production occurs in a contraction-dependent manner and the magnitude of increase is directly proportional to the intensity, duration, and amount of muscle mass recruited during exercise (118,119). While a consistent increase in IL-6 has been reported following exhaustive and prolonged exercise (i.e. marathon running) (120-123), exercise intensity may be more important during short-duration bouts (124-127).

For instance, a two-fold increase in plasma IL-6 has been observed following as little as 6-min of "all out" rowing exercise (2 min x 3 bouts) in trained males (126). Another well-designed investigation examining the cytokine response to graded exercise among active males

reported greater post-exercise IL-6 concentrations following 60 min of running at 75% VO_{2max} compared to equal duration bouts at 55% and 65% VO_{2max} (127). The influence of exercise intensity was confirmed in another study involving knee extension exercise performed by healthy males at either 65% or 85% of maximal power output, with positive correlations between exercise intensity and IL-6 release from human thigh muscle (125). A recent examination of the IL-6 response to HIIT (5 x 4 min cycling at 80% VO_{2max}, 3 min intervals at 50% VO_{2max}) revealed a significantly greater increase in IL-6 (~2.5-fold) compared to a MICT bout (~1.5-fold) of the same duration (35 min cycling at 50% VO_{2max}), which was positively related to the mean exercise VO_2 (124). Collectively, elevations in IL-6 following acute exercise seem to be intensity dependent with a graded response observed with increasing exercise intensity.

Potential role in appetite-regulation

Cytokines such as IL-6 have long been implicated in the control of feeding particularly during the manifestation of anorexia and/or cachexia under pathological conditions (128-130). Though their ability to modulate peripheral (i.e. gastrointestinal activity) and central (i.e. neuropeptide release) under such conditions has been established, only recently has their role in post-exercise feeding been investigated (128-130). The link between IL-6 and post-exercise energy intake was demonstrated in a recent study by Almada and colleagues (131) who reported a 2.6-fold increase in plasma IL-6 with concomitant suppression of ad libitum post-exercise energy intake in young males compared to resting controls following treadmill running (45 min at 60% VO_{2max} followed by 7 min at 90% VO_{2max}). Although concentrations of appetite-regulating hormones were not measured, elevations in systemic IL-6 concentrations following 90 min of treadmill running to exhaustion have been shown to coincide with a 2.5-fold increase in circulating GLP-1 in rodents (132). This rise in GLP-1 following exercise was absent in IL-6

knockout mice and when IL-6 action was blocked (132). Furthermore, incubation of the intestinal L cell line GLUTag with increasing concentrations of IL-6 has been shown to increase GLP-1 secretion in a dose-dependent manner (132). These observations are consistent with other investigations that support IL-6's role in stimulating peripheral GLP-1 release in humans and mediating its anorexigenic actions in mice via GLP-1 receptor activation in the CNS (133,134). This IL-6 dependent mechanism may also extend to PYY given that both GLP-1 and PYY have a common secretory origin (intestinal L-cells) and an increase in PYY mRNA expression has been observed in mice following IL-6 injection (132). In addition to its peripheral effects, IL-6 and its receptor are highly expressed in the hypothalamus and have been shown to modulate neuropeptide release in key appetite-regulatory nuclei (135-137). Thus, IL-6 has also been implicated in mediating the central action of various appetite-regulatory signals (134,138-140).

Taken together, evidence suggests that exercise-induced changes in IL-6 appear to be driven by the intensity at which the activity is performed, particularly during brief duration exercise bouts. Furthermore, IL-6 appears to alter appetite-regulating hormone release and action in animals, and has been linked to decreased post-exercise energy intake in humans. Given that the appetite-regulatory response to an acute exercise bout may be intensity-dependent, IL-6 could provide an important mechanistic link between exercise intensity and appetite-regulation.

Gaps in the literature:

1. No study to date has employed exercise intensity as a model to increase IL-6 while concurrently examining changes in appetite-related parameters (i.e. gastrointestinal hormones, appetite perceptions).
2. The exclusion of HICT from previous comparisons between exercise protocols has made it difficult to establish a clear dose-response relationship between exercise intensity and appetite regulation.

Research objectives:

1. To utilize exercise intensity during submaximal continuous (i.e. MICT and HICT) and sprint interval running as a method to increase plasma IL-6 and examine its association with post-exercise alterations in acylated ghrelin, active glucagon-like peptide-1 and appetite perceptions.
2. To establish a clear dose-response relationship between exercise intensity and appetite regulation.

Hypotheses:

1. IL-6 concentrations will increase in an intensity dependent manner and coincide with post-exercise changes in appetite-related parameters.
2. Changes in appetite-regulating hormones and appetite perceptions will also occur in an intensity-dependent manner that favors a greater suppression of appetite with increasing exercise intensity.

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CHAPTER 2:

MANUSCRIPT

The effects of exercise intensity on acylated ghrelin, active glucagon-like peptide-1 and appetite perceptions: examining the potential involvement of interleukin-6

1. Abstract

Context: Interleukin-6 (IL-6) stimulates the release of appetite-regulating hormones in animals and associates with decreased energy intake in humans. Thus, IL-6 may contribute to the intensity-dependent effects of exercise on appetite-related parameters.

Objective: To examine the effects of exercise intensity on IL-6, appetite-regulating hormones, and appetite perceptions.

Design: Eight males completed four sessions: 1) Moderate-intensity continuous training (MICT; 30 min running, 65% VO_{2max}); 2) High-intensity continuous training (HICT; 30 min running, 85% VO_{2max}); 3) Sprint interval training (SIT; 4 x 30 sec "all-out" running bouts separated by 4 min recovery); 4) Control (CTRL; no exercise). Blood samples were obtained immediately pre- and post-exercise, as well as 30- and 90-min post-exercise for the measurement of acylated ghrelin, active glucagon-like peptide-1 (GLP-1), and IL-6. Appetite perceptions were assessed at the same time-points using a visual analog scale. Energy intake was recorded for a 3-day period starting from the day preceding each session.

Results: Acylated ghrelin and appetite were suppressed after HICT ($P<0.005$) and SIT ($P<0.002$), though more so after SIT compared to MICT ($P<0.042$). Active GLP-1 concentrations increased immediately after MICT ($P<0.001$) and 30 min after HICT ($P<0.001$) and SIT ($P=0.005$). Changes in IL-6 coincided with decreases in acylated ghrelin and correlated negatively with appetite after HICT. Simultaneous increases in GLP-1 and IL-6 were observed 30 min after HICT and SIT. Energy intake was reduced on the day after HICT ($P<0.03$).

Conclusions: These findings support an intensity-dependent paradigm for appetite-regulation that is strongly associated with changes in acylated ghrelin and may be mediated by IL-6.

2. Introduction

Effectively combatting the current obesity epidemic requires an improved understanding of energy balance, which involves the interplay between energy intake and energy expenditure (1). While seemingly unsophisticated, the complex physiological, psychological, and environmental inputs that influence energy balance have made it difficult to manipulate its components effectively (2). As such, attempts to reduce body mass through dietary restriction or isolated exercise are often ineffective potentially due compensatory increases in energy intake that prevent the energy deficit required for weight loss (1,3-5). Clearly, there is an urgent need for interventions that simultaneously target both components of energy balance in a manner that improves body composition.

High-intensity interval training (HIIT) involves brief near maximal bouts of exercise interspersed with periods of rest or low-intensity activity (6). Sprint interval training (SIT) is a more intense form of intermittent exercise traditionally structured as four to six 30-sec supramaximal (>100% VO_{2max}) bouts followed by 4-min recovery periods (6). Both HIIT and SIT promote rapid physiological adaptations and, more importantly for energy balance, can also reduce fat mass despite significantly lower training volume and time commitment than moderate-intensity (50-75% VO_{2max}) continuous training (MICT) (7-9). Typically, these effects have been attributed to elevations in post-exercise metabolism that increase energy expenditure and/or fat utilization in hours after HIIT and SIT (10-14). However, recent evidence suggests that these protocols may also facilitate fat loss through acute modifications in appetite and/or energy intake, making them a valuable tool for offsetting recent obesogenic trends (15-17).

The physiological regulation of energy intake involves complex interactions between key brain regions involved in energy homeostasis and peripheral organs/tissues that secrete hormones

with orexigenic (appetite-stimulating) or anorexigenic (appetite-inhibiting) properties (18). Acute energy intake is primarily influenced by episodic signals released from the gut that detect short-term energy status and influence appetite through perceptions of hunger and satiety (19). The only peripheral signal with orexigenic properties is the peptide hormone ghrelin, which is released from endocrine cells in the stomach during periods of energy deficit (i.e. fasting) (20). The initial product is converted to its biologically active (acylated) form by the enzyme ghrelin O-acyl transferase (GOAT) (21). Acting in opposition to ghrelin is the incretin product of intestinal L-cells, glucagon-like peptide-1 (GLP-1), which exerts anorexigenic effects in response to nutrient ingestion (22). The two active forms of GLP-1 ($GLP\text{-}1_{7\text{-}36}$ and $GLP\text{-}1_{7\text{-}37}$) are rapidly degraded to the inactive $GLP\text{-}1_{9\text{-}36}$ by the enzyme dipeptidyl peptidase-4 (DPP-IV) (23).

Acute exercise (30-90 min) leads to decreases in acylated ghrelin with simultaneous increases in GLP-1 (active and total) that facilitate reductions in short-term appetite and/or energy intake (15,16). Furthermore, these changes may be intensity-dependent leading to potentially greater suppression of appetite and/or energy intake following high-intensity exercise protocols such as HIIT and SIT (17). However, as most studies to date have involved MICT there is no clear consensus regarding the effects of exercise intensity on appetite-related parameters (15). Despite recent comparisons between MICT and HIIT/SIT, the exclusion of high-intensity (>75% VO_{2max}) continuous training (HICT) has made it difficult to establish a clear dose-response relationship between exercise intensity and appetite-regulation ((24-27).

Similarly, the mechanisms underlying exercise-induced changes in appetite-regulating hormones are unclear, though several lines of evidence support an intensity-dependent paradigm for appetite regulation (17). A particularly attractive potential mechanism involves the

contraction mediated cytokine interleukin-6, which increases in an intensity-dependent manner following an acute exercise bout (28,29). The link between IL-6 and post-exercise energy intake was demonstrated in a recent study that reported increased plasma IL-6 concentrations with concomitant reductions in ad libitum energy intake in young males following treadmill running (30). Though appetite-regulating hormones were not measured in this study, systemic increases in IL-6 through direct infusion or exhaustive exercise have been shown to stimulate GLP-1 secretion from murine intestinal L-cells (31,32). Furthermore, this GLP-1 response is abolished in IL-6 knockout mice or when IL-6 action is blocked, suggesting a regulatory role for IL-6 in GLP secretion (31-33). The effects of IL-6 on appetite and food intake may also be mediated centrally via direct actions on key neuronal circuits involved in energy homeostasis, though its effects on other peripheral signals such as ghrelin are currently unknown (34-38). Also, the applicability of these findings to humans is limited, as no study to date has concurrently examined changes in IL-6 and appetite-regulating hormones following exercise. Given that the appetite-regulatory response to an acute exercise bout may be intensity-dependent, IL-6 could provide an important mechanistic link between exercise intensity and appetite regulation.

The primary purpose of this study was to examine the effects of exercise intensity using running-based MICT, HICT, and SIT on changes appetite-regulating hormones (acylated ghrelin and active GLP-1), appetite perceptions and IL-6. We hypothesized that increases in IL-6 would be proportional to exercise intensity, and coincide with intensity-dependent changes acylated ghrelin, active GLP-1 that favor decreases in appetite and/or energy intake.

3. Methods

Participants

Eight active young males volunteered to participate in this study. Participants were non-smokers and healthy as assessed by the PAR-Q health questionnaire (Appendix C). All participants were physically active (≤ 3 weekly exercise sessions), though none were involved in a systemic training program nor had they been for at least 4 months prior to data collection. Participants were not taking any dietary supplements at the time of the study. Experimental details were fully explained to all participants and all provided written informed consent prior to any data collection (consent form shown in Appendix D). This study was approved by the Research Ethics Board at Wilfrid Laurier University in accordance with the 1964 Declaration of Helsinki.

Study design

Participants completed four experimental sessions (~3 h each) in a systematic rotational order with each session performed ≥1 week apart. Experimental sessions consisted of one resting control session (CTRL; no exercise) and three exercise sessions involving running-based protocols: 1) MICT (65% VO_{2max}); 2) HICT (85% VO_{2max}); 3) SIT (brief bouts of "all-out" running interspersed with short recovery periods). Blood samples and subjective appetite measures were obtained at several time-points during each session. Participants were instructed to refrain from physical activity, alcohol, and caffeine for ≥24 h before each experimental session. Energy intake was recorded for a 3-day period and participants were asked to replicate their dietary intake for 24 h prior to each session.

Pre-experimental procedures

All participants completed one familiarization session (1 week) prior to the first experimental session to introduce testing procedures/equipment and reduce any learning effects during subsequent sessions. During this session, participants also performed a graded exercise test to exhaustion on a motorized treadmill (4Front, Woodway, WI, USA) for the determination of maximal oxygen consumption (VO_{2max}). Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was measured continuously using an online breath-by-breath gas collection system (MAX II, AEI technologies, PA, USA) that was calibrated using known gas concentrations and a 3-L syringe for flow. Each participant wore a fitted silicon facemask (7400 series Vmac, Hans Rudolph Inc. KS, USA) ensuring comfort and preventing leaking during gas measurements. Heart rate (HR) was recorded beat-to-beat using an integrated HR monitor (FT1, Polar Electro, QC, Canada). After a 5-min treadmill warm-up, each participant ran at a self-selected pace (5-7 mph) that was maintained throughout the test, with incremental increases in workload (2% grade) applied every 2 min until volitional fatigue. VO_{2max} was defined as the greatest 30-second average at which VO_2 values (<1.35 $ml \cdot kg^{-1} \cdot min^{-1}$) plateaued despite increases in workload, or two of the following criteria: 1) respiratory exchange ratio (RER) value >1.10; 2) maximal HR (within 10 bpm of age-predicted maximum [220-age]) and/or; 3) voluntary exhaustion. All participants achieved the RER and HR criteria used for the determination of VO_{2max}, while six out of eight participants also achieved a plateau in VO_2 values. Following a 5-min treadmill cool-down and a short rest period (>20 min), the running speed/grade required to elicit appropriate workloads for the MICT (65% VO_{2max}) and HICT (85% VO_{2max}) sessions were determined. Briefly, participants began jogging at a moderate pace

(5 mph) with incremental increases in speed (1-2 mph) applied every 3 min (to achieve steady state) hereafter, until the speed corresponding to the appropriate workload was achieved and recorded. Participants were then allowed to practice "all-out" running efforts on a specialized self-propelled treadmill (HiTrainer Pro, QC, Canada) on which all SIT sessions were performed. Speed indices from this treadmill (i.e. peak, minimum, and average) have been validated against the traditional Wingate anaerobic test that is typically utilized during repeated sprint exercise (Islam et al. in preparation).

Experimental sessions

Participants arrived at the laboratory at 0800 h after an overnight fast and remained in the laboratory for the next ~3 h (Figure 1). Upon arrival participants were given a standardized test meal consisting of a Chocolate Chip Clif Bar (68% carbohydrates, 17% fat, 15% protein) and water (provided ad libitum throughout session). The appropriate amount of Clif bar (g) was weighed on a digital scale to provide an intake of 7 kcal/kg body mass. Based on this requirement, participants consumed 545.8±55.9 kcal or 147.9±15.1 g (2.2±0.2 bars). The test meal was to be consumed within ~15 min after which participants rested quietly while sitting for 30 min. Exercise commenced at 0850 h and consisted of a 5-min standardized warm-up (3.5 mph), a 30 min running-based protocol (18 min for SIT with an additional 12 min of rest prior to warm-up to match protocol duration), and a 5-min cool-down. Gas exchange (VO_2 and VCO_2) and HR were measured continuously during exercise using the gas collection system and integrated HR monitor described previously. Upon completion of exercise (0930 h), participants rested quietly (reading or using laptop) for an additional 90 minutes. Venous blood samples were obtained at 0845 h (pre-exercise), 0930 h (immediately post-exercise), 1000 h (30 min post-exercise), and 1100 h (90 min post-exercise). Perceptions of appetite were assessed at the same

blood sampling time-points using a visual analog scale (39). Identical procedures were followed during the CTRL session with the exception of the exercise period (0850-0930 h) during which participants rested quietly.

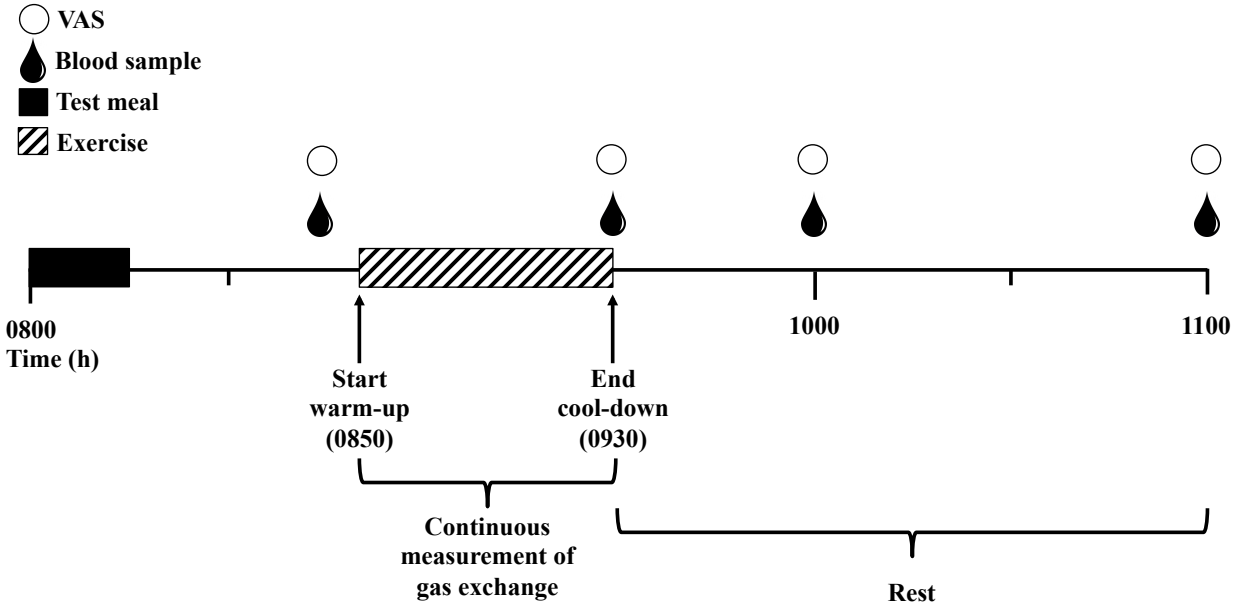


Figure 1. Experimental session timeline. VAS: visual analog scale.

Exercise protocols

The MICT and HICT protocols were performed on a motorized treadmill (4Front, Woodway, WI, USA) and consisted of 30 minutes of continuous running at a target workload of 65% and 85% VO_{2max}, respectively. Participants began running at the pre-determined work rate corresponding to the target intensity and VO_2 was continuously monitored to adjust work rate as needed to maintain intensity (using speed/grade adjustments). The SIT protocol was performed on a self-propelled sprint treadmill (HiTrainer Pro, QC, Canada) and consisted of four, 30-second "all out" running efforts interspersed with 4-min rest periods. Participants were instructed to exert maximal effort for the entire duration of each sprint and strong verbal encouragement was provided throughout.

Blood processing and analysis

All blood samples were collected by venipuncture from the antecubital vein while participants were in a supine position for the measurement of IL-6, acylated ghrelin, active GLP-1 (GLP-1_{7-36} and GLP-1_{7-37}). Two samples (3 mL whole blood each) were collected into separate pre-chilled Vacutainer tubes coated with K2 EDTA (5.4 mg) at each time-point. 40 μL of AEBSF (25 mg/mL) per mL whole blood was added to one of the tubes to prevent degradation of acylated ghrelin whereas 10 μL of DPP-IV inhibitor and 500 KIU aprotinin per mL whole blood were added to the other tube to prevent inactivation of GLP-1. All tubes were gently inverted 10 times and centrifuged at 3000 g for 10 min. The plasma supernatant was then dispensed into Eppendorf tubes while the plasma from the ghrelin Vacutainer was acidified by the addition of 100 μL of 1 M HCl per mL plasma. All plasma supernatant was stored at -80°C for subsequent analysis. Commercially available enzyme-linked immunosorbent assay kits were used to determined plasma concentrations of acylated ghrelin (Cat. # EZGRA-88K, EMD Millipore, MA, USA), active GLP-1 (Cat. # EGLP-35K, EMD Millipore, MA, USA) and IL-6 (Cat. # D6050, R&D Systems, MN, USA) according to the manufacturer's instructions. The acylated ghrelin assay was specific for measuring the active (acylated) form of this peptide in human serum or plasma with no cross reactivity to inactive (des-acyl) ghrelin. The active GLP-1 assay was specific for measuring the two active forms of this peptide (GLP-1_{7-36} and GLP-1_{7-37}) in human plasma with no cross reactivity with other forms of GLP-1 (i.e. GLP-1_{1-36}, GLP-1_{1-37}, GLP-1_{9-36}, GLP-1_{9-37}). The IL-6 assay was specific to natural and recombinant human IL-6 with no cross-reactivity with other cytokines. All samples were run in duplicate and were batch

analyzed for each participant to eliminate inter-assay variation. The intra-assay coefficients of variations for acylated ghrelin, active GLP-1 and IL-6 were 8.3, 9.1 and 4.8 %, respectively.

Appetite perceptions

Appetite perceptions were assessed using a visual analog scale (VAS) shown in Appendix E that has been previously validated for quantifying appetite perceptions (39). Perceptions of hunger (i.e. "How hungry do you feel?"), satisfaction (i.e. "How satisfied do you feel?"), fullness (i.e. "How full do you feel?") and prospective food consumption (i.e. "How much do you think you can eat?") were reported on a 100 mm scale anchored at each end with contrasting statements (i.e. "not at all" and "extremely"). The mean values of the four appetite perceptions were used to calculate an overall appetite score after inverting the values for satisfaction and fullness (40).

Energy Intake

Free-living energy intake was recorded for a 3-day period (starting on the day before each experimental session) using self-reported dietary logs (Appendix F). Participants were provided a copy of their dietary intake on the day prior to their first experimental session and were asked to replicate their intake on the day prior to all subsequent sessions. Given the known limitations of using self-reported dietary logs, a 24-h recall was also conducted during follow-up interviews (on the morning of each session and within 48 h after each session) to verify the accuracy of the self-reported food intake (41). Furthermore, participants were provided detailed instructed (including a sample log) pertaining to ensure proper measurement and recording.

Statistical Analysis

All data were analyzed using Sigma Stat for Windows (Version 3.5). Due to the individual variability in absolute hormone concentrations and appetite perceptions, changes at

each time point were expressed relative to each participant's baseline values as described previously (42). Absolute hormone concentrations and appetite scores can be found in Appendix A and B, respectively. All area under the curve (AUC) calculations for blood-related parameters and appetite perceptions were performed using the trapezoid method. One-way repeated measures analysis of variance (ANOVA) was used to compare absolute hormone concentrations at baseline, AUC values across sessions, and ad libitum energy intake. Two-way repeated measures ANOVA (session x time) was used to compare differences in IL-6, appetite-regulating hormones, and appetite perceptions across each time-point in all experimental sessions. Tukey's HSD tests were used for post-hoc analysis where necessary. Relationships between variables were assessed using Pearson product-moment correlations. Significance was set at $P<0.05$. All data are presented as mean±standard deviation (SD).

4. Results

Participant characteristics

Participants were 23.1±3.0 years of age with a mean VO_{2max} of 51.2±4.4 mL·kg^{-1}·min^{-1} (4.01±0.27 L·min^{-1}) and the following physical characteristics: height: 178.2±2.7 cm; weight: 78.7±8.1 kg; BMI: 24.8±2.3 kg/m^2.

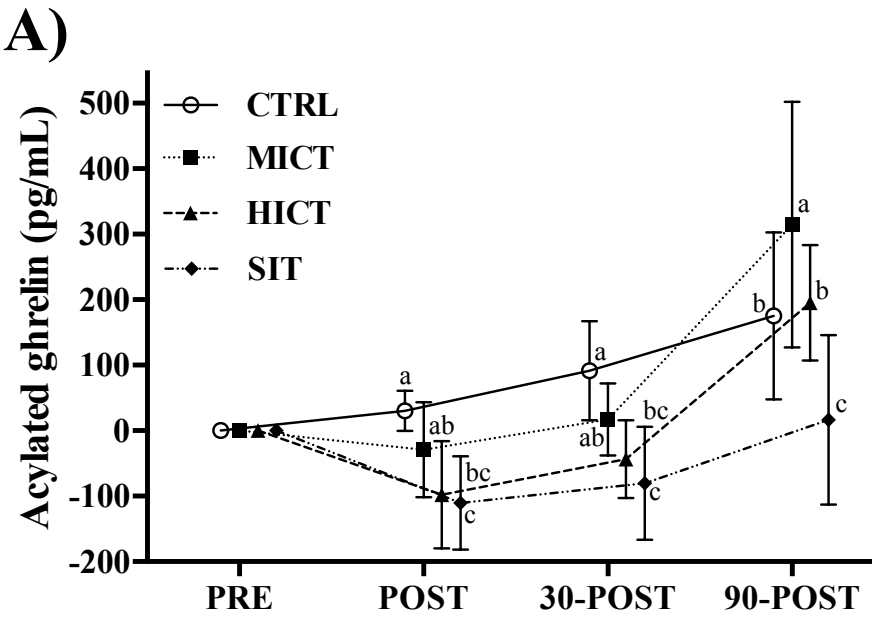
Exercise responses

The 30-min MICT and HICT were completed at work rate corresponding to 2.64±0.19 L·min^{-1} (65.9±1.9% VO_{2max}) and 3.29±0.22 L·min^{-1} (82.0±0.8% VO_{2max}), respectively. Average HR during MICT and HICT was 161±12 bpm and 178±18 bpm, respectively. The 18-min SIT protocol elicited an average VO_2 of 1.47±0.09 L·min^{-1} (56.0±5.6% VO_{2max}) with a mean HR of 130±13 bpm (rest periods inclusive). Respiratory exchange ratio values during MICT, HICT,

and SIT were 0.98±0.05, 1.04±0.03, 1.41±0.07, respectively. VO_2-derived estimates of energy expenditure (5 kcal/L O_2) were 396.1±28.8, 492.8±33.4 and 132.3±8.2 kcal for MICT, HICT and SIT, respectively.

Acylated ghrelin

There were no differences (P=0.524) in absolute acylated ghrelin concentrations at baseline (CTRL: 193.9±114.5 pg/mL; MICT: 216.2±164.6; HICT: 231.1±185.2; SIT: 250.0±214.2). Two-factor ANOVA revealed a significant (P<0.001) interaction (session x time) for changes in acylated ghrelin concentrations relative to baseline (Fig 2A). Specifically, acylated ghrelin was suppressed immediately and 30 min post-exercise after both HICT (P<0.005) and SIT (P<0.002) compared to CTRL, while MICT had no effect (P>0.177). SIT also elicited lower acylated ghrelin concentrations at 30 min post-exercise versus MICT (P=0.043) and at 90 min post-exercise vs. all other sessions (P<0.001). Acylated ghrelin concentrations at 90 min post-exercise were not different between HICT and CTRL (P=0.949), though both were lower compared to MICT (P<0.009). AUC values for acylated ghrelin (Fig 2B) were not different between sessions (P>0.271).



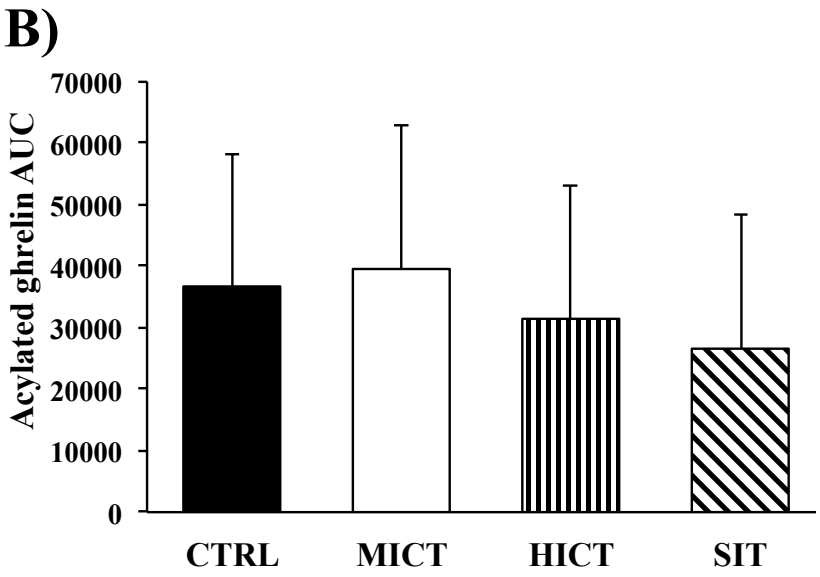


Figure 2. Changes in acylated ghrelin across all time-points relative to baseline (A) and area under the curve (AUC) values for acylated ghrelin (B). *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. Unlike letters indicate between-session differences at each time-point ($P<0.05$).

Active GLP-1

There were no differences ($P=0.925$) in absolute active GLP-1 concentrations at baseline (CTRL: 8.46±2.45 pM; MICT: 7.95±2.06; HICT: 7.71±2.28; SIT: 8.23±2.54). Two-factor ANOVA revealed a significant ($P<0.001$) interaction (session x time) for changes in active GLP-1 concentrations relative to baseline (Fig 3A). Active GLP-1 concentrations were elevated immediately post-exercise after MICT compared to all other sessions ($P<0.001$), while HICT and SIT were both unchanged at this time point ($P>0.760$). Active GLP-1 concentrations were increased at 30 min post-exercise after both HICT ($P<0.001$) and SIT ($P=0.005$) compared to CTRL, with the difference between MICT and CTRL approaching significance ($P=0.059$). HICT also elicited higher GLP-1 concentrations at this time point compared to both MICT ($P<0.001$) and SIT ($P=0.002$), which were not different from each other ($P=0.836$). Only HICT ($P=0.032$) and SIT ($P=0.010$) remained elevated at 90 min post-exercise versus CTRL, though

both were similar compared to MICT (P>0.811). AUC values for active GLP-1 (Fig 3B) were not different between sessions (P>0.155).

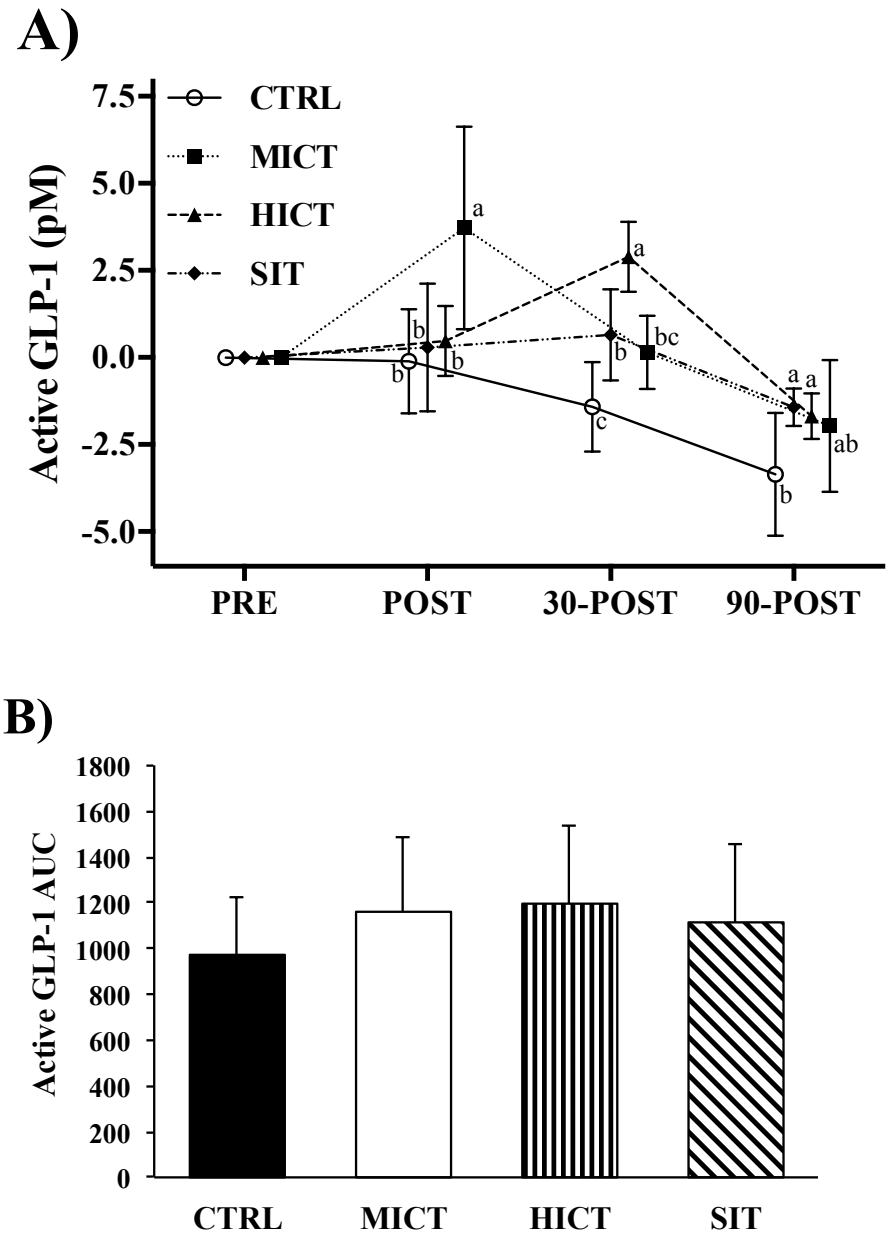


Figure 3. Changes in active GLP-1 across all time points relative to baseline (A) and area under the curve (AUC) values for active GLP-1 (B). *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. Unlike letters indicate between-session differences at each time-point (P<0.05).

IL-6

There were no differences (P=0.301) in absolute IL-6 concentrations at baseline (CTRL: 1.61±0.27 pg/mL; MICT: 1.41±0.26; HICT: 1.57±0.29; SIT: 1.37±0.35). Two-factor ANOVA revealed a significant (P<0.001) interaction (session x time) for changes in IL-6 concentrations relative to baseline (Fig 4A). IL-6 concentrations were increased immediately post-exercise after HICT (P=0.002) versus CTRL, with the increase after SIT also approaching significance (P=0.087), though no effect was observed after MICT (P=0.457). IL-6 was increased at 30 min post-exercise during all exercise sessions versus CTRL (P<0.001), though to a greater extent after HICT compared to MICT (P=0.007). IL-6 concentrations at this time point were not different between HICT and SIT (P=0.268) or between SIT and MICT (P=0.419). IL-6 concentrations remained elevated at 90 min post-exercise after HICT compared to CTRL (P=0.015), while also approaching significance versus SIT (P=0.062), with no other between session differences at this time point (P>0.391). AUC values for IL-6 (Fig 4B) were significantly greater during HICT compared to both MICT (P=0.017) and CTRL (P=0.002). A trend suggesting higher AUC values during both MICT (P=0.062) and SIT (P=0.060) compared to CTRL was also observed.

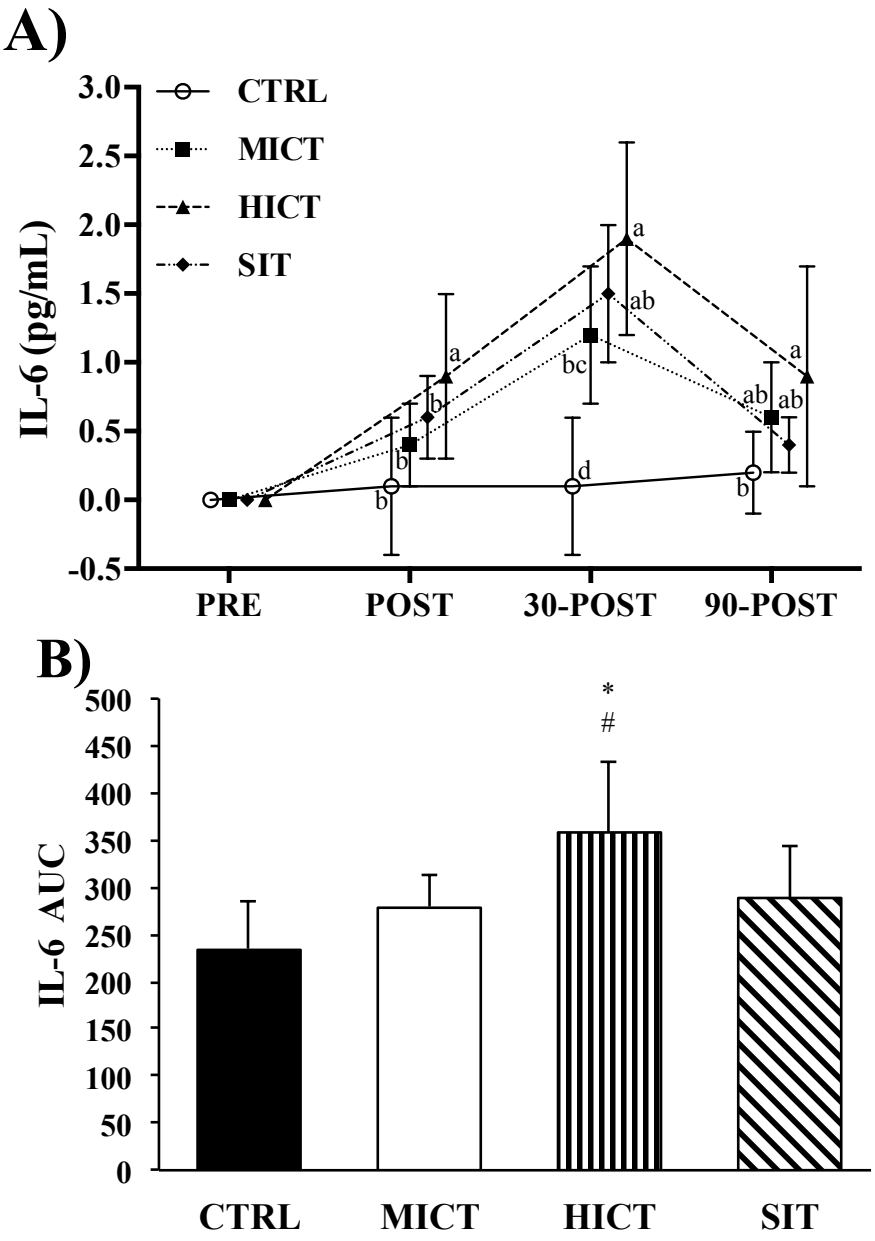


Figure 3. Changes in IL-6 across all time points relative to baseline (A) and area under the curve (AUC) values for IL-6 (B). *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. Unlike letters indicate between-session differences at each time-point; * significantly different versus CTRL; # significantly different versus MICT ($P<0.05$).

Appetite perceptions

There were no differences in absolute VAS scores pertaining to hunger (P=0.506), satisfaction (P=0.916), fullness (P=0.950), prospective food consumption (P=0.905) and overall appetite (P=0.750) at baseline. Significant interactions (session x time) were observed for changes in perceptions of hunger (P<0.001), satisfaction (P=0.038), fullness (P=0.005), prospective food consumption (P<0.001) and overall appetite (P<0.001) relative to baseline.

Hunger (Fig 5A) was suppressed immediately post-exercise compared to CTRL (P<0.033), though to a greater extent after SIT compared to MICT (P=0.043) and also trending to be greater after HICT (P=0.078). Hunger was also suppressed at 30 min post-exercise after HICT and SIT compared to both MICT (P<0.013) and CTRL (P<0.001). Hunger remained suppressed at 90 min post-exercise after SIT compared to all other sessions (P<0.006).

Satisfaction (Fig 5B) increased immediately post-exercise after HICT (P=0.006) and SIT (P<0.001) versus CTRL, and at 30 min post-exercise after SIT (P<0.001) with the increase after HICT also approaching significance (P=0.074). Only SIT resulted in increased satisfaction at 90 min post-exercise compared to CTRL (P=0.044).

Perceived fullness (Fig 5C) was increased immediately post-exercise after both SIT (P<0.001) and HICT (P=0.001) versus CTRL, though only SIT was increased compared to MICT (P=0.049). Fullness remained higher at 30 min post-exercise after both HICT (P=0.004) and SIT (P=0.006) compared to CTRL, and at 90 min post-exercise after IT compared to both MICT (P=0.035) and CTRL (P=0.003).

Prospective food consumption (Fig 5D) decreased immediately post-exercise versus CTRL (P<0.014), though to a greater extent after SIT compared to MICT (P=0.012). This effect persisted to 30 min post-exercise after HICT (P=0.014) and SIT (P<0.001) versus CTRL, and

also after SIT compared to MICT (P=0.023). Only SIT resulted in lower prospective food consumption at 90 min post-exercise compared to CTRL (P=0.002).

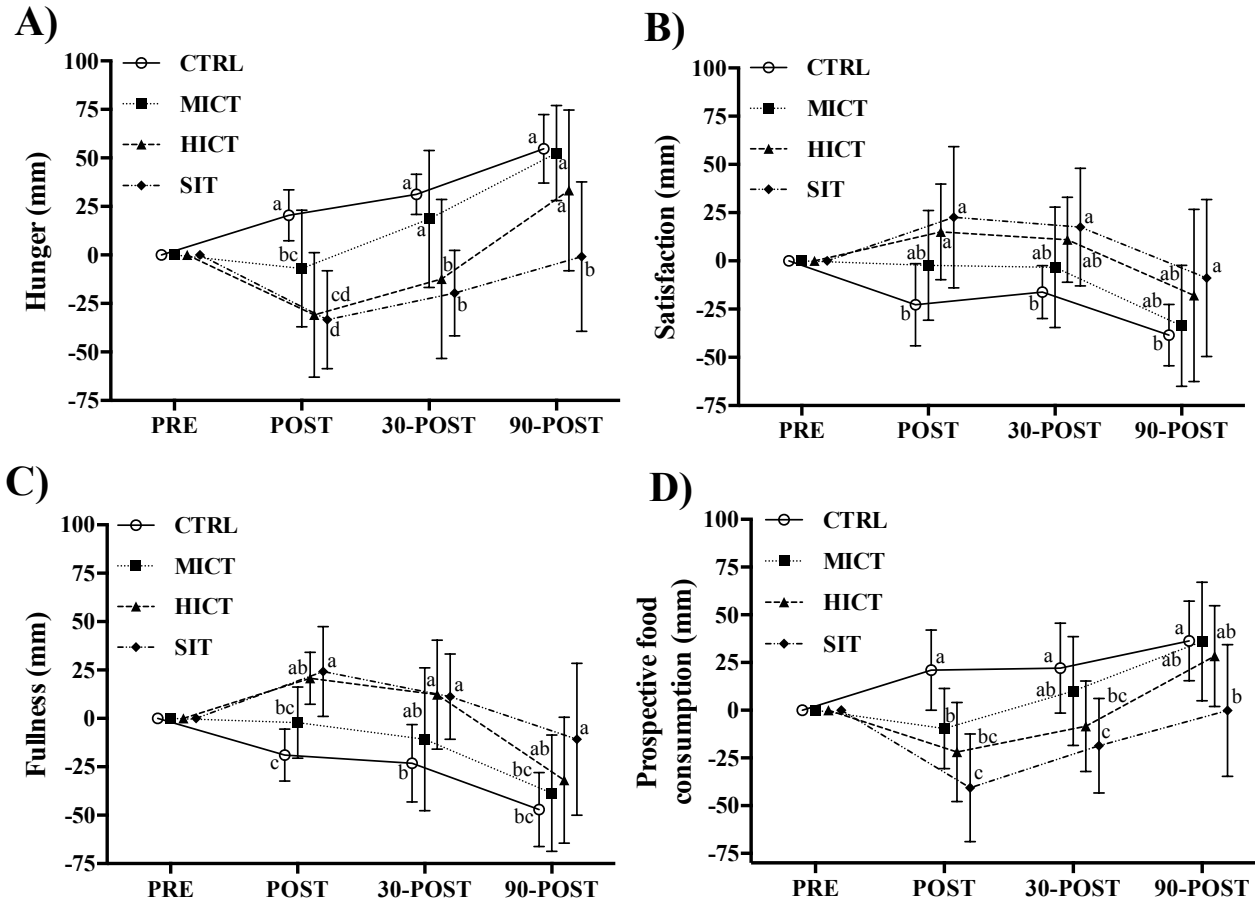


Figure 5. Changes in the perception of hunger (A), satisfaction (B), fullness (C), and prospective food consumption (D) across all time points relative to baseline. *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. Unlike letters indicate between-session differences at each time-point (P<0.05).

Overall appetite (Fig 6A) decreased immediately post-exercise (P<0.034), though to a greater extent after SIT compared to MICT (P=0.012). Appetite was also suppressed at 30 min post-exercise after HICT (P=0.001) and SIT (P<0.001) compared to CTRL, and after SIT compared to MICT (P=0.010). Appetite remained suppressed at 90 min post-exercise after SIT compared to all other sessions (P<0.041). AUC values for overall appetite (Fig 6B) were lower

during SIT compared to CTRL (P=0.033). A trend suggesting lower AUC values during HICT compared to CTRL (P=0.066) and during SIT compared to MICT (P=0.062) was also observed.

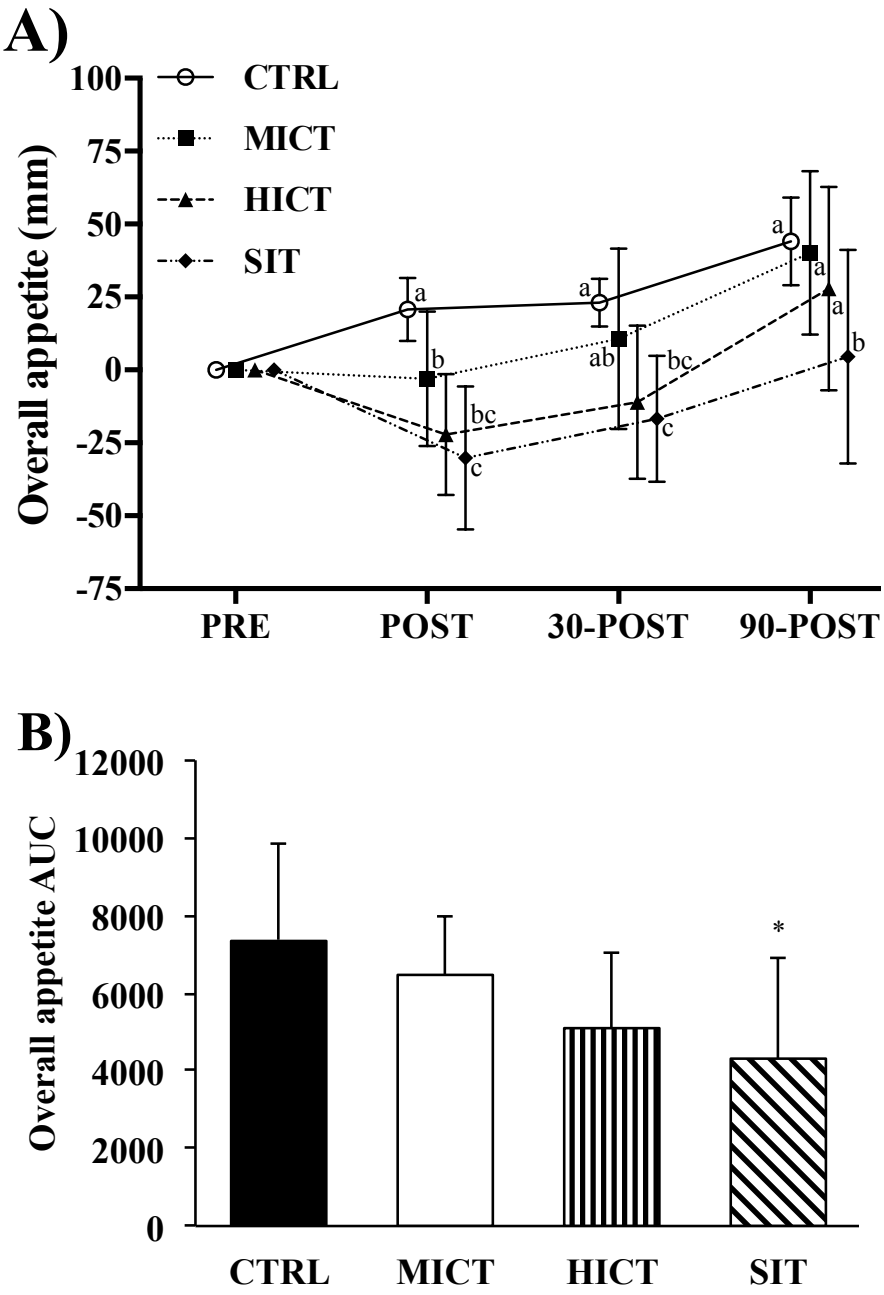


Figure 6. Changes in overall appetite across all time points relative to baseline (A) and area under the curve (AUC) values for overall appetite (B). *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. Unlike letters indicate between-session differences at each time-point; * significantly different versus CTRL (P<0.05).

Energy intake

As intended, there were no differences (P=0.987) in energy intake on the day prior to the experimental session (Table 1). There were also no significant (P>0.112) differences in free-living energy intake on the day of the experimental session (Fig 7A). Energy intake on the day after the experimental session (Fig 7B) was significantly lower after HICT compared to both MICT (P=0.028) and CTRL (P=0.020), while a trend (P=0.053) suggesting lower energy intake on the day after SIT compared to both MICT (P=0.063) and CTRL (P=0.053) was also observed.

Table 1. Energy and macronutrient intake on the day prior to each experimental session.

	CTRL	MICT	HICT	SIT
Total energy (kcal)	2104.1±410.6	2072.6±421.3	2070.5±464.3	2088.0±439.7
Carbohydrate (g)	270.3±85.4	254.2±101.0	263.9±110.8	259.9±78.8
Carbohydrate (%)	50.6±7.5	47.6±9.2	49.6±8.8	49.3±7.0
Fat (g)	67.8±12.8	70.0±13.3	64.9±9.0	67.5±12.0
Fat (%)	29.4±5.4	31.0±6.6	28.9±5.0	29.7±5.3
Protein (g)	103.1±23.2	105.0±22.0	108.9±32.7	110.4±35.2
Protein (%)	20.0±5.3	20.9±5.4	21.8±7.0	21.1±5.3

Note: CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training.

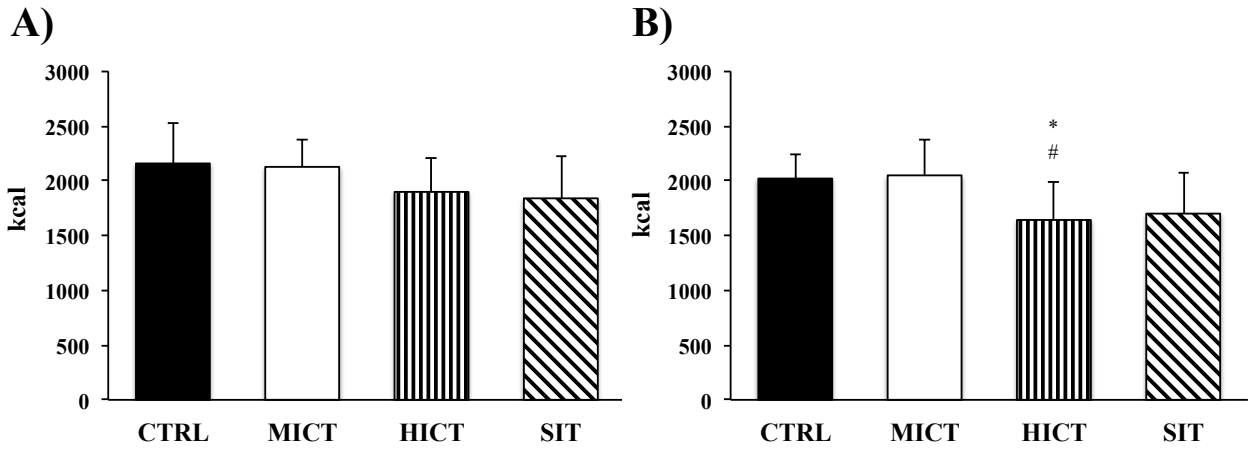


Figure 7. Total energy intake on the day of (A) and the day after (B) each experimental session. *Note:* CTRL: control; HICT: high-intensity continuous training; MICT: moderate-intensity continuous training; SIT: sprint interval training. * significantly different versus CTRL; # significantly different versus MICT (P<0.05).

Correlations

There were no significant correlations between IL-6 and appetite-regulating hormones (Table 2), though the correlation between IL-6 and acylated ghrelin at immediately (r = -0.55, P=0.078) and 30 min post-exercise (r = -0.58, P=0.066) approached significance during the SIT session. Acylated ghrelin was positively correlated (r = 0.72, P=0.022) with overall appetite at the 90 min post-exercise in the HICT session (Table 3). There was also a negative correlation between IL-6 and perceptions of overall appetite (30 min post-exercise: r = -0.70, P=0.027; AUC: r = -0.69, P=0.029) in the HICT trial (Table 3).

Table 2. Correlations between IL-6 and appetite-regulating hormones.

	IL-6				
	Pre-exercise	**Imm post-exercise**	**30 min post-exercise**	**90 min post-exercise**	**AUC**
GLP-1					
CTRL	0.30	-0.08	0.25	0.56[a]	0.29
MICT	-0.34	-0.43	0.17	-0.46	-0.44
HICT	0.70*	-0.04	0.08	-0.21	0.14
SIT	0.09	-0.36	-0.25	0.21	-0.19
Acylated ghrelin					
CTRL	-0.32	-0.32	0.05	0.20	0.19
MICT	-0.53	-0.15	0.21	-0.53	-0.25
HICT	0.12	0.11	0.37	-0.32	0.12
SIT	-0.34	-0.55[a]	-0.58[a]	-0.24	-0.50

Note: Values in table are Pearson's correlation coefficients (r). CTRL: control; HICT: high-intensity continuous training; Imm: immediately; MICT: moderate-intensity continuous training; SIT: sprint interval training. * P<0.05, [a] P<0.08 (trend observed).

Table 3. Correlations between blood parameters and overall appetite perceptions.					
	OVERALL APPETITE				
	Pre-exercise	**Imm post-exercise**	**30 min post-exercise**	**90 min post-exercise**	**AUC**
GLP-1					
CTRL	-0.25	-0.31	-0.47	-0.11	-0.41
MICT	0.03	0.54	0.39	-0.29	0.25
HICT	-0.11	0.41	-0.06	0.13	0.17
SIT	-0.38	0.22	0.07	0.17	0.01
Acylated ghrelin					
CTRL	-0.86*	-0.88*	-0.71*	-0.44	-0.78*
MICT	-0.32	0.17	0.23	-0.11	-0.02
HICT	-0.41	0.10	0.26	0.72*	0.16
SIT	-0.49	0.11	0.27	0.48	0.19
IL-6					
CTRL	0.31	-0.24	-0.42	-0.25	-0.28
MICT	0.42	-0.40	0.16	0.22	-0.32
HICT	0.20	-0.48	-0.70*	-0.52	-0.69*
SIT	-0.09	-0.26	-0.28	-0.51	-0.34
Note: Values in table are Pearson's correlation coefficients (r). CTRL: control; HICT: high-intensity continuous training; Imm: immediately; MICT: moderate-intensity continuous training; SIT: sprint interval training. * $P<0.05$.					

5. Discussion

To our best knowledge, this is the first study to concurrently examine appetite-regulatory and IL-6 responses to running-based MICT, HICT, and SIT. Both high-intensity protocols suppressed acylated ghrelin for 30 min post-exercise (while MICT had no effect) and this suppression persisted to 90 min post-exercise after SIT only. While MICT induced a rapid increase in active GLP-1 immediately post-exercise, an increase was observed 30 min after HICT and was greater than SIT. Appetite was suppressed immediately post-exercise after all three protocols (more so after SIT versus MICT) and remained suppressed for 30 min post-exercise after HICT and SIT, and for 90 min post-exercise after SIT only. In accordance with

appetite suppression, free-living EI on the day following exercise was reduced after HICT and also appeared to be lower after SIT (trend observed). Post-exercise increases in IL-6 also occurred in an intensity-dependent manner showing an inverse correlation with appetite after HICT. IL-6 concentrations peaked at 30 min post-exercise and coincided with peak GLP-1 concentrations during the HICT and SIT sessions only. These findings support an intensity-dependent paradigm for appetite-regulation, which appears to be strongly associated with changes in acylated ghrelin. While IL-6 appears to contribute to the anorexic response after high-intensity exercise, its relationship with peripheral hormone concentrations remains to be fully elucidated.

The suppression of acylated ghrelin after both high-intensity protocols in our study supports a clear intensity-dependent response for this hormone. Though the effects of HICT have not been previously investigated, the majority of studies consistently demonstrating post-exercise decreases in acylated ghrelin have involved more strenuous ($\geq$70% VO_{2max}) versions of MICT (43-48). At lower exercise intensities (<70% VO_{2max}) longer duration exercise bouts may be necessary to suppress acylated ghrelin (25,49,50) though some studies have failed to show an effect (27,51,52). As such, exercise-induced suppression acylated ghrelin may be dependent on reaching an intensity threshold, particularly during short duration exercise bouts as highlighted by the present results. Interestingly, acylated ghrelin concentrations increased in a compensatory manner after MICT, and were highest at 90 min post-exercise compared to all other sessions, highlighting the potential for lower intensity exercise to stimulate post-exercise appetite and/or energy intake (2,4,53). Contrarily, only SIT elicited lower acylated ghrelin concentrations compared to MICT and remained lower at 90 min post-exercise compared to all other sessions. While only four studies to date have examined acylated ghrelin responses to repeated sprint

exercise, all have demonstrated acute suppression compared to resting values (25-27,54). In accordance with our findings, two of these studies have also demonstrated a greater effect compared to MICT (27,54), though we extend these findings by showing a sustained suppression of acylated ghrelin following SIT. It is possible that the supramaximal and/or intermittent nature of SIT may promote metabolic perturbations unique to this type of exercise that influence both the magnitude and duration of acylated ghrelin suppression.

In contrast to acylated ghrelin, the effects of exercise intensity on active GLP-1 were less clear. Specifically, MICT induced a rapid increase in active GLP-1 immediately after exercise, while HICT and SIT elicited a delayed response at 30 min post-exercise, which was greater after HICT. Examination of GLP-1 responses to acute exercise is limited to date and the majority of studies have involved MICT, while only a few have measured the active forms (GLP-$1_{7\text{-}36}$, GLP-$1_{7\text{-}37}$) of this peptide (26,49,55-58). The immediate post-exercise GLP-1 response after MICT is in agreement with previous studies reporting rapid increases in both total and active GLP-1 following similar intensity (50-75% VO_{2max}) exercise protocols (26,55-58). While the effects of HICT on this peptide have not been investigated, increasing MICT intensity from 50 to 75% VO_{2max} failed to promote greater increases in active GLP-1 suggesting that lower exercise intensities sufficiently stimulate its release (58). Given that the initial (0-30 min post-exercise) increase in GLP-1 in our study was of similar magnitude between MICT and HICT (albeit at different time-points) and more robust in comparison with SIT, it is possible that exercise duration (i.e. energy expenditure) may be important for inducing rapid increases in this peptide. However, GLP-1 concentrations remained elevated at 90 min post-exercise after both HICT and SIT while MICT had returned to resting values, resulting in an overall similar GLP-1 response to exercise. As such, despite marked differences in exercise energy expenditure (MICT: 396 kcal;

HICT: 493 kcal; SIT: 133 kcal) that promote rapid increases in GLP-1 after both HICT and MICT, protracted increases in resting energy expenditure that occur after intense interval exercise likely contribute to similar GLP-1 responses over the entire post-exercise period (11-13). Accordingly, similar post-exercise increases in total GLP-1 have been previously reported following MICT and modified short-duration SIT expending ~250 and ~125 kcal, respectively (26).

In agreement with the concept of "exercise-induced anorexia" (59), we observed an acute suppression of appetite that was characterized by decreased perceptions of hunger and prospective food consumption with concomitant increases in perceived satisfaction and fullness. We also observed clear effects of exercise intensity, as overall appetite remained suppressed for 30 min after both HICT and SIT, and for 90 min after SIT only. Furthermore, the suppression of appetite after SIT was greater than MICT at all time points, and also greater than HICT at 90 min post-exercise. As such, exercise intensity appears to influence both the magnitude and duration of this response, with more profound effects observed after supramaximal interval exercise. These findings are in support of previous studies reporting appetite suppression after strenuous ($\geq$70% VO_{2max}) endurance exercise (44,45,59). Though the examination of appetite responses to intensities above 80% VO_{2max} has been limited to intermittent exercise, our findings extend those of Deighton and colleagues (24,25) who reported greater appetite suppressive effects of HIIT and SIT compared to lower intensity (<70% VO_{2max}) versions of MICT, though observed compensatory increases in appetite (but not energy intake) in the hours after SIT. While others have also reported heightened appetite perceptions after MICT that would be expected to stimulate energy intake in the hours after exercise, we did not observe such a compensatory response after any exercise protocol within the acute experimental time frame of the current

study (44,53). In fact, we observed a decrease in free living energy intake on the day after the HICT session (and a trend suggesting lower intake on the day after SIT), which extends previous reports of suppressed 24 h energy intake following high-intensity exercise in overweight/obese populations, though we are the first to report this in active young males (27,60). While we acknowledge the limitations associated with self-reported dietary intake (41), our data combined with previous findings support the ability of high-intensity protocols such as HIIT and SIT to suppress perceptions of appetite that can facilitate reductions in energy intake (24,25,27,61,62).

As expected, IL-6 concentrations increased in an intensity-dependent manner peaking at 30 min post-exercise, with the magnitude of increase being greater after HICT compared to MICT, though similar to SIT. As low muscle glycogen stimulates IL-6 mRNA expression and release, a greater reliance on carbohydrate metabolism (as evidence by the RER values) explains the greater response after HICT compared to matched duration MICT (28,29,63). This also likely explains the ability of SIT to increases IL-6 despite the drastically reduced exercise duration, as repeated sprint exercise significantly depletes muscle glycogen stores (64). The overall IL-6 response coincided with intensity-dependent reductions in acylated ghrelin and the negative correlation between the two parameters approached significance during the SIT session (30 min post-exercise: $r = -0.58$, $p=0.065$). Although simultaneous peaks in IL-6 and active GLP-1 concentrations were observed during the HICT session, the two were not correlated in the post-exercise period making it difficult to establish a clear relationship between these variables such as that observed previously in animals (31-33). It is possible that IL-6 response in our study was of insufficient magnitude to influence the secretion of this peptide as previously reported increases in GLP-1 were preceded by substantially greater (>100-fold) increases in systemic IL-6 (31). The high aerobic fitness of the participants in our study may have also have played a role

as the IL-6 response to exercise may be blunted in trained individuals (76). Interestingly, we did observe a negative correlation between the IL-6 concentrations and overall appetite during the HICT trial (30 min post-exercise: r = -0.70, P=0.027; AUC: r = -0.69, P=0.029) highlighting the potential for this cytokine to influence appetite and/or energy intake (30). Apart from peripheral effects, accumulating evidence suggests that the effects of IL-6 on energy homeostasis are also mediated centrally via direct actions in key hypothalamic nuclei (35,37). Consistent with the hypothalamic expression of IL-6 and its receptor, intracerebroventricular IL-6 administration reduces energy intake and body weight in animals while IL-6 deficiencies lead to the development of mature-onset obesity (38,65). Furthermore, central IL-6 interacts with the same neuronal circuits (NPY/AgRP, POMC) by which peripheral signals exert their orexigenic/orexigenic effects (34,36). While these findings support the role of IL-6 in appetite regulation, its proposed role as a regulator of peripheral GLP-1 secretion remains to be established in humans.

Though IL-6 provides one potential mechanism by which exercise intensity influences appetite-related parameters there are several other possibilities that are also likely to contribute, particularly after high-intensity exercise. For instance, it has been frequently hypothesized that exercise-induced reductions in splanchnic blood flow to accommodate the demands active musculature may interfere with the secretion and/or clearance of acylated ghrelin (17,44). Additionally, as exercise consistently reduces acylated (but not total or unacylated) ghrelin, which also decreases in hypoxic environments, impaired oxygen delivery to the gut may interfere with the enzymatic activity of GOAT hereby disrupting the acylation process (21,50,66). On the other hand, changes in GLP-1 may be mediated by increases in circulating catecholamines and/or free fatty acids (FFA) as activation of adrenergic and G protein-coupled FFA receptors on

intestinal L-cells has been shown to stimulate their secretory activity (67-70). Other key metabolites such as lactate, glucose, and insulin that are elevated immediately after intense exercise have also been shown to inhibit appetite and/or energy intake potentially through peripheral effects on appetite-regulating hormones as well as central actions in the hypothalamus (27,71-75). As such, the greater appetite-suppressive effects of high-intensity exercise are likely attributable to a combination of these factors rather than one mechanism alone.

While the current study provides valuable information regarding the effects of exercise intensity on acute appetite-regulation, several limitations should be highlighted. First, given the nature of the study it was not possible to establish a causal relationship between IL-6 and appetite-regulating hormones such as that reported in animals using IL-6 injection or IL-6 knockout models. Secondly, the acute experimental time frame of this may not have fully encapsulated the magnitude and/or duration of the appetite-regulatory response to exercise, limiting our ability to observe any protracted effects. Although we did attempt to quantify free-living energy intake through self-report dietary records, we acknowledge the limitations (i.e. underestimation of intake, measurement errors) associated with such techniques, though these have shown to be of greater concern in overweight/obese populations (41). Third, the measurement of PYY_{3-36} would have allowed for a more comprehensive examination of the anorexigenic response to exercise, particularly since this peptide may be more responsive to exercise intensity than GLP-1 (58). Finally, the small sample size and the participant characteristics (active, normal weight, young males) used in this study limit the applicability of our findings to overweight/obese, sedentary, and/or female populations.

6. Conclusion

Taken together, the findings of this study support an intensity-dependent paradigm for appetite-regulation, which appears to be closely associated with changes in acylated ghrelin. Contrarily, exercise intensity does not seem to influence active GLP-1, suggesting that the anorexic effects of high-intensity exercise may more consequential to decreases in acylated ghrelin. Though IL-6 also increases in in an intensity-dependent manner correlating inversely with appetite, its relationship with peripheral concentrations of appetite-regulating hormones in humans requires further examination. Nevertheless, these findings add to the growing body of literature supporting the ability of HIIT and SIT to improve energy balance through simultaneous effects on energy expenditure and energy intake, making them a valuable tool in the battle against obesity.

7. Acknowledgements

We would like to thank all participants for their time and effort. This study was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (TJH) and a Wilfrid Laurier University Category A Internal Grant (TJH).

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CHAPTER 3:

SUMMARY, CONCLUSIONS, & FUTURE DIRECTIONS

The current study examined the effects of exercise intensity on appetite-related parameters while also investigating a potential mechanism that may mediate these effects. The results of this investigation provide valuable insight into the acute appetite-regulatory response to exercise leading to several important conclusions. First, by comparing a traditional moderate-intensity exercise protocol to both continuous and intermittent forms of high-intensity exercise, we were able to establish a clear dose-response relationship between exercise intensity and appetite-regulation. Specifically, we found that both high-intensity protocols suppressed appetite to a greater extent than moderate-intensity activity, and that these changes were accompanied by intensity-dependent reductions in acylated ghrelin, which is a potent appetite-stimulant. Second, we found that exercise intensity had little influence on active GLP-1, which increased similarly after both moderate- and high-intensity exercise. Third, we found that intensity-dependent increases in IL-6 do not appear to influence active GLP-1 concentrations as previously reported in animals. However, given that changes in IL-6 coincided with reductions in appetite and acylated ghrelin, our findings do in fact support the role of this cytokine in appetite-regulation. While these findings improve our understanding of the mechanisms involved in the acute regulation of energy intake, they also raise several important points that must be highlighted.

The ability of exercise to elicit acute hormonal responses that favor reductions in appetite and/or energy intake clearly contradicts the homeostatic nature of energy regulation (1). Given that dietary restriction often stimulates compensatory increases in appetite and/or energy intake while exercise may not, indicates that different methods of imposing an energy deficit lead to highly divergent outcomes (2,3). Furthermore, as moderate-intensity exercise is sometimes associated with similar compensatory responses to those observed after food restriction, the stimulus arising from this type of exercise is likely insufficient for disrupting energy sensing

mechanisms, thus allowing them to rapidly restore energy balance (4-6). Contrarily, the metabolic perturbations associated with more intense protocols likely pose a greater challenge to the restoration of physiological equilibrium as evidenced by the prolonged metabolic effects that persist in the hours after HIIT and SIT (7-9). As such, the greater appetite-suppressive effects of high-intensity exercise (at least over an acute time-period) may arise from its ability to disrupt and/or override the homeostatic mechanisms that maintain energy balance.

While exercise intensity clearly seems to be important for suppressing acylated ghrelin concentrations and overall appetite, its dissociation with the active GLP-1 response warrants some speculation. As alluded to in the manuscript, reductions in gastric blood flow have been frequently proposed to mediate the suppression of acylated ghrelin (via disruption of GOAT activity) following intense exercise (10-12). Based on this logic, it could be argued that the delayed and/or attenuated active GLP-1 response after intense exercise is a consequence the peptide's inability to enter circulation. However, given that PYY_{3-36} has been shown to increase in an intensity-dependent manner, this hypothesis seems unlikely as both peptides have a common secretory origin (intestinal L cells) and are co- expressed/released (13,14). Alternatively, the divergent effects of exercise intensity on these two peptides may be explained by changes in the enzymatic activity of DPP-IV, which metabolizes both GLP-1 and PYY albeit in different ways. Specifically, DPP-IV facilitates the conversion of PYY_{1-36} to the biologically active PYY_{3-36}, while the two active forms of GLP-1 ($GLP\text{-}1_{7-36}$ and $GLP\text{-}1_{7-37}$) are degraded to the inactive $GLP\text{-}1_{9-36}$ by the same enzyme (13). Though speculative, it is possible that exercise intensity has direct effects on the activity of this enzyme leading to increases in circulating PYY_{3-36} while concurrently decreasing active GLP-1 concentrations in plasma due to its increased degradation to $GLP\text{-}1_{9-36}$. Though this mechanism may potentially explain the

intensity-dependent increases in PYY_{3-36} but not active GLP-1, the effects of exercise on the enzymatic activity of DPP-IV have yet to examined.

It is important to emphasize that the peripheral signals measured in the current study (and the majority of the literature) represent only a portion of the neuroendocrine loop that governs energy homeostasis (Chapter 1, Figure 1). Given that the effects of appetite-regulating hormones and various other peripheral signals are ultimately mediated by changes in hypothalamic neuropeptides (NPY, AgRP, POMC, CART), elucidation of these central mechanisms is imperative for achieving a thorough understanding of appetite-regulation (15,16). While a regulatory role of IL-6 in mediating peripheral hormone release was only recently investigated, this cytokine has long been implicated in feeding particularly during disease-induced anorexia (18). Furthermore, accumulating evidence highlights its ability to increase energy expenditure, reduce energy intake, and lower body weight when administered centrally (17-20). Given that its involvement in appetite regulation may be more evident at the level of the CNS, systemic increases in IL-6 following exercise may influence appetite and/or energy intake by directly altering the activity of key neuronal populations (21-23). Currently, there is no direct evidence for this effect and the majority of work examining the involvement of these neuropeptides in feeding has involved animal models. Thus, examination of the effects of exercise on hypothalamic neuropeptides highlights an important venue for future research.

Finally, given the acute nature of the current study, it is important to extend this work by examining long-term energy balance following high-intensity exercise protocols. While the examination of acute alterations in appetite and peripheral hormones provides important insight into the immediate anorexic effects of exercise, improvements in body composition are ultimately facilitated by sustained and repeated periods of energy deficit. It has been

documented that reductions in body weight alter peripheral hormones in a manner that defends body stores and favor the regain of body weight (24). For instance, elevated levels of acylated ghrelin with concurrent decreases in PYY and the long-term adiposity signal leptin have been reported after a weight loss period, all of which would be expected to stimulate appetite and lead to potential increases in energy intake (25,26). As such, while high-intensity protocols such as HIIT/SIT may acutely perturb energy homeostasis in a manner that suppresses appetite and/or energy intake, their effects on long-term energy balance remain to be examined. Presently, only one study has examined the long-term effects of HIIT on appetite regulation (in overweight individuals) and found little effects on circulating hormone concentrations (27).

To conclude, our findings support an intensity-dependent paradigm for appetite-regulation while also highlighting the potential involvement of IL-6. Furthermore, our findings raise several important questions that may help guide future research:

1) What are the effects of exercise intensity on the enzymatic activities of GOAT and DPP-IV and their subsequent contribution to peripheral hormone concentrations?
2) How does exercise intensity influence concentrations of hypothalamic neuropeptides and what is their association with peripheral changes in appetite-regulating hormones?
3) Are the effects of IL-6 on post-exercise appetite and/or energy intake mediated by changes in peripheral hormone concentrations or central interactions with key neuropeptides?
4) What are the effects of high-intensity protocols on long-term changes in appetite-regulating hormones, particularly after weight reduction?

Collectively, these questions will improve our mechanistic understanding of both short- and long-term energy balance and ultimately lead to more effective strategies for weight management. Based on the current findings and the supporting evidence provided, it indeed appears that high-intensity exercise is superior to moderate intensity activity for inducing an acute energy deficit through appetite suppression as well as increased energy expenditure. Thus, in addition to their well-documented health and performance benefits, protocols such as HIIT and SIT provide viable strategies for improving body composition and combatting the obesity epidemic.

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Appendix A

Table 1. Absolute concentrations of acylated ghrelin, active GLP-1, and IL-6 at each time-point during all experimental sessions.

	Pre-exercise	Imm post-exercise	30 min post-exercise	90 min post-exercise
Acylated ghrelin (pg/mL)				
CTRL	193.9±114.5	224.0±127.4	285.5±176.1	369.3±232.3
MICT	216.2±164.6	187.2±115.5	260.5±150.1	530.7±323.6
HICT	231.1±185.2	133.0±112.2	187.9±142.3	426.1±246.2
SIT	250.5±214.2	140.2±156.0	170.5±135.4	266.8±201.0
GLP-1 (pM)				
CTRL	8.46±2.45	8.48±2.12	6.91±2.26	5.10±1.14
MICT	7.95±2.06	11.67±3.72	8.09±1.93	5.99±2.79
HICT	7.71±2.28	8.18±2.54	11.88±3.60	6.02±2.03
SIT	8.23±2.54	7.85±2.82	9.56±2.60	6.80±2.26
IL-6 (pg/mL)				
CTRL	1.61±0.27	1.73±0.48	1.71±0.53	1.85±0.44
MICT	1.41±0.26	1.85±0.32	2.63±0.31	1.96±0.39
HICT	1.57±0.29	2.49±0.74	3.46±0.75	2.45±0.85
SIT	1.37±0.35	2.01±0.59	2.89±0.55	1.74±0.32

Note: CTRL: control; HICT: high-intensity continuous training; Imm: immediately; MICT: moderate-intensity continuous training; SIT: sprint interval training.

Appendix B

Table 1. Absolute VAS scores (mm) for hunger, satisfaction, fullness, prospective food consumption and overall appetite at each time-point during all experimental sessions.

	Pre-exercise	Imm post-exercise	30 min post-exercise	90 min post-exercise
Hunger				
CTRL	15.9±18.4	36.3±24.1	47.1±20.1	70.6±12.4
MICT	28.1±25.9	21.1±12.2	46.7±14.8	80.7±13.4
HICT	37.4±32.3	6.6±7.4	25.1±17.5	70.7±16.8
SIT	45.1±32.7	11.9±15.7	25.4±27.5	44.3±23.4
Satisfaction				
CTRL	63.7±21.9	41.0±15.0	47.6±28.0	25.3±16.9
MICT	61.6±32.7	59.3±23.4	58.3±24.2	28.0±22.2
HICT	57.0±28.5	72.1±32.0	68.0±29.6	39.1±19.6
SIT	54.3±33.0	76.9±31.9	71.9±26.6	45.4±25.1
Fullness				
CTRL	66.4±23.6	47.6±29.5	43.3±31.8	19.3±14.8
MICT	63.1±31.1	61.0±19.8	52.4±21.9	24.6±22.3
HICT	58.6±29.3	79.3±23.8	70.9±18.1	26.7±18.3
SIT	59.9±31.3	84.1±21.0	71.1±24.5	49.1±27.2
Prospective food consumption				
CTRL	38.7±25.3	59.9±19.3	60.7±23.8	75.0±20.0
MICT	41.7±29.5	32.1±15.2	51.9±16.7	77.7±13.9
HICT	43.7±24.1	21.9±27.7	35.3±26.5	72.1±12.2
SIT	48.6±29.7	8.0±7.3	30.0±30.5	48.4±23.3
Overall appetite				
CTRL	31.1±19.4	51.9±21.5	54.3±23.8	75.3±13.9
MICT	36.3±29.0	33.3±13.7	47.0±13.7	76.5±15.5
HICT	41.4±26.7	19.3±21.2	30.4±19.6	69.3±12.8
SIT	44.9±30.5	14.7±17.6	28.1±25.5	49.5±24.2

Note: CTRL: control; HICT: high-intensity continuous training; Imm: immediately; MICT: moderate-intensity continuous training; SIT: sprint interval training.

Appendix C

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT _____ WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

Appendix D

CONSENT TO PARTICIPATE IN RESEARCH
LETTER OF INFORMATION

Date:

Title of Study: ***The effects of graded exercise intensity on plasma concentrations of interleukin-6 and appetite-regulating hormones***

Dear _____:

You are being invited to participate in a research study conducted by Tom J. Hazell (PhD), Hashim Islam (BSc Kin), and Logan Townsend (BA Kin) from the Energy Metabolism Research Laboratory.

PURPOSE OF THE STUDY
The primary purpose of this study is to determine the effect of exercise intensity on energy intake. More specifically we will examine plasma concentrations of interleukin-6 and appetite-regulating hormones following treadmill running performed at two different intensities.

PROCEDURES
This study requires you to visit the Energy Metabolism Research Laboratory 4 times, once for a familiarization session (<1 h) and then for 4 testing sessions (~3.5 h each) for a total time commitment of 14.5 hours. Experimental sessions (separated by 1 week) will include either a rest period or a 40 min exercise session (5 min warm-up & cool-down + 30 min exercise) followed by a 2 h post-exercise measure of gas exchange and blood measurements where participants rest comfortably and quietly in the laboratory (i.e. reading). The 4 testing sessions will be: 1) 30 min running at 65% of maximal oxygen consumption; 2) 30 min running at 85% of maximal oxygen consumption; 3) Sprint interval training (4 x 30 s sprints with 4 min rest in between); 4) No exercise (resting quietly for 30 min). All exercise sessions include only 2-30 min of actual exercise. You will also be asked provide 4 blood samples (collected by trained personnel) pre-exercise, immediately post-exercise, as well as 30- and 90-minutes post-exercise, from the forearm. You will also be asked questions pertaining to feelings of hunger, satiety, and desire to eat at the same time-points as the blood draws. You will also be asked to record all physical activity and dietary intake over a 3-day period using logs.

POTENTIAL RISKS AND DISCOMFORTS
There is a possibility of mild muscle soreness and/or fatigue typical of an exercise session. Although phlebotomy is safe when done by certified and training individuals there is a small risk of bruising at the puncture site which can be reduced by keeping pressure on the site for several minutes after the needle is withdrawn. In some rare cases the vein may become inflamed after the sample is withdrawn however this can be alleviated by using a warm compress. There is a small risk of infection any time the skin is broken however this rarely occurs when equipment is properly sterilized and disposed of. Some people may also experience light-headedness if they are uncomfortable with needles and if this occurs the experiment will be terminated immediately. The risk of falling if this occurs is minimum as the participant will be seated in a secure phlebotomy chair.

POTENTIAL BENEFITS TO SUBJECTS AND/OR SOCIETY

The potential benefits of your participation in this study include an improvement in your exercise capacity as well as a better understanding of your cardiorespiratory fitness. Results from this study will further our understanding of high-intensity exercise effects appetite-regulating hormones and post-exercise food intake.

CONFIDENTIALITY

All information obtained in connection with this study will be de-identified. All contact information is collected and stored on a master list in a password-protected file with access to only the study investigators. All participants will be assigned an arbitrary number to ensure anonymity. This study number will be used in all data collection files and mean data will be stored in a password protected file for comparison with future studies. Raw data will not be released to any other parties and all results will be collapsed before analysis. If you wish to withdraw from the study all personal information will be removed from our records and any obtained blood samples will be destroyed.

PARTICIPATION AND WITHDRAWAL

Your participation in this research study is completely voluntary. If you are a student and volunteer to be in this study, you may withdraw at any time without any effect on your status at Wilfrid Laurier University. If you are not a student, you may withdraw at any time. You may also refuse to answer any questions you feel are inappropriate and still remain in the study. The investigators may withdraw you from this research if circumstances arise which warrant doing so (i.e. lack of effort during exercise sessions, difficulty scheduling, repeatedly missing scheduled sessions, etc.).

FEEDBACK OF THE RESULTS OF THIS STUDY

If you would like a copy of a lay summary of the results please check the box below. The results from this study will be reported in general terms in the form of speech or writing that may be represented in manuscripts submitted for publication in scientific journals, or oral and/or poster presentations at scientific meetings, seminars, and/or conferences. We plan to publish this study in an academic journal. The information published in a journal or subsequent studies will not identify you in any way. Copies will be available upon request.

☐

SUBSEQUENT USE OF DATA

This de-identified data may be used in subsequent studies (with no link to your personal information). You will receive a copy of the consent form after it has been signed and do not waive any legal rights by signing it.

This letter is yours to keep. If you have any questions about this research project feel free to call:
Dr. Tom Hazell 519-884-1970 x3048

Further, if you have any questions about the conduct of this study or your rights as a research subject you may contact Dr. R. Basso, Research Ethics Board (REB) Chair (rbasso@wlu.ca / 519-884-0710 x4994).

Sincerely,

Hashim Islam (isla9020@mylaurier.ca), MSc Student
Logan Townsend (town9000@mylaurier.ca), MSc Student
Dr. Tom Hazell (thazell@wlu.ca), Assistant Professor

Department of Kinesiology and Physical Education
Wilfrid Laurier University

Title of Study: ***The effects of graded exercise intensity on plasma concentrations of interleukin-6 and appetite-regulating hormones***

Consent Statement

Principal Investigators: Dr. Tom Hazell

I have read the accompanying "Letter of Information" and have had the nature of the study and procedures to be used explained to me. All of my questions have been answered to my satisfaction.

By signing below, I agree to participate in this study

NAME (please print): _____

SIGNATURE: _____

DATE: _____

NAME OF PERSON OBTAINING INFORMED CONSENT (please print):

_____

SIGNATURE OF PERSON OBTAINING INFORMED CONSENT:

_____

DATE: _____

Appendix E

How hungry do you feel?

Not hungry at all	_____	**Very hungry**

How satisfied do you feel?

I am completely empty	_____	**I cannot eat another bite**

How full do you feel?

Not full at all	_____	**Very full**

How much do you think you can eat?

Nothing at all	_____	**A lot**

Appendix F

Daily Food Log

Instructions:

1. Record all food intake for a 3 day period (day before session, day of session, day after session)
2. Try to consume foods that you would typically eat as part of your regular diet.
3. Keep your recording sheets with you at all times. (Snacks are typically consumed unpredictably and, as a result, it is impossible to record them accurately unless your recording forms are nearby.)
4. Use a small food scale if you have one or standard-measuring devices (measuring cups, measuring spoons, etc) to record the quantities consumed, as accurately as possible. If you do not eat all of the item re-measure what's left and record the difference.
5. Record combination foods separately (i.e., hot dog, bun, and condiments) and include brand names of food items (list contents of homemade items) whenever possible.
6. For packaged items, use labels to determine quantities.

Example:

Time of Day (i.e. 8:15 am, 12:30 pm)	**Food Item** (include brand name if possible)	**Quantity** (i.e. g, mL, cups, etc.)	**Notes** (i.e. ingredients & amounts used if possible)
9:30 am	Eggs	2 whole	½ tsp salt, ½ cup cheese, ½ tsp butter
9:30 am	Egg whites	½ cup	-
10:15 am	Tropicana orange juice	1 cup	-
11:05 am	Apple	1 whole	-
1:50 pm	Dominos Pizza	4 slices	Pepperoni, mushroom, cheese
1:50 pm	Pepsi	500 ml	-

DAY 1 (day before session) Date: _____

Time of Day (i.e. 8:15 am, 12:30 pm)	**Food Item** (Include brand name if possible)	**Quantity** (i.e. g, mL, cups, etc.)	**Notes** (i.e. ingredients & amounts used if possible)

Appendix G

Energy Metabolism Research Laboratory Dept. of Kin. & Phys. Ed.

EXERCISE INTENSITY & APPETITE STUDY

PURPOSE: To determine the effects of exercise intensity on blood hormones involved in appetite regulation

WHO?

- Healthy individuals (aged 18-35 years) who are recreationally active

WHAT?

- 5 laboratory visits
  - One familiarization session (<1 h)
  - Four experimental sessions (3.5 h each)
- Pre- and post-exercise measurements of oxygen consumption and blood hormones

WHEN?

- Sessions start at 8 am on a day that you are available!

WHERE?

- Energy Metabolism Research Laboratory (NC106)

WHY?

- Get your cardiorespiratory fitness (VO_{2MAX}) assessed for free
- Learn more about how exercise affects appetite and food intake
- Use specialized equipment not available in most facilities

If interested please contact:

Hashim Islam, M.Sc. student – isla9020@mylaurier.ca
Dr. Tom Hazell – thazell@wlu.ca

THIS STUDY HAS BEEN APPROVED BY THE RESEARCH ETHICS BOARD (REB#4596).