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EXAMINING THE POTENTIAL INVOLVEMENT OF SEX HORMONES ON APPETITE REGULATION IN FEMALES ACROSS THE MENSTRUAL CYCLE

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ABSTRACT

Appetite regulation has exclusively been examined during the follicular phase (FP) of the menstrual cycle, and it remains unclear whether the greater levels of estradiol (E_2) and progesterone (P_4) in the luteal phase (LP) affect this process. Thus, the purpose of this study was to utilize vigorous-intensity continuous training (VICT) as a method to modulate plasma concentrations of E_2 and P_4 and examine their associations with post-exercise alterations in acylated ghrelin, peptide tyrosine-tyrosine$_{3\text{-}36}$ ($PYY_{3\text{-}36}$), active glucagon like peptide-1 (GLP-1), subjective appetite perceptions, and energy intake across the menstrual cycle. Eleven women completed 30 min of VICT (80% $\dot{V}O_{2max}$) in both the FP and LP in a randomized order. E_2, P_4, acylated ghrelin, $PYY_{3\text{-}36}$, active GLP-1, and appetite perceptions were measured pre-, immediately, 30, and 90 min post-exercise, and energy intake was recorded for a 3-day period beginning the day prior to each experimental session. E_2 concentrations increased immediately post-exercise (P=0.041) with no differences in P_4 (P=0.078). Acylated ghrelin was significantly lower in the FP compared to the LP (P=0.029), and active GLP-1 was elevated at 30 min post-exercise compared to 90 min post-exercise (P=0.016). There were no observable effects of exercise or menstrual cycle phase on $PYY_{3\text{-}36}$ (P=0.305), overall appetite (P=0.616), or free-living energy intake (P=0.244). These findings suggest the typical post-exercise suppression of acylated ghrelin may be blunted in the presence of elevated sex hormones in the LP, though sex hormones do not appear to play a role in the anorexigenic hormone response to acute exercise. Future work should focus on the reproducibility of our findings to further elucidate the influence of sex hormones on appetite regulation across the menstrual cycle.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1: LITERATURE REVIEW

CHAPTER 2: MANUSCRIPT

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

CHAPTER 2: MANUSCRIPT

LIST OF ABBREVIATIONS

AEBSF: 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
AgRP: Agouti-related peptide
ARC: Arcuate nucleus
CART: Cocaine and amphetamine-regulated transcript
CCK: Cholecystokinin
CSEP: Canadian Society for Exercise Physiology
DPP-IV: Dipeptidyl peptidase-4
E_2: Estradiol
EDTA: Ethylenediaminetetraacetic acid
ELISA: Enzyme-linked immunosorbent assay
FSH: Follicle-stimulating hormone
FP: Follicular phase
GAQ: Get Active Questionnaire
GLP-1: Glucagon-like peptide 1
GnRH: Gonadotropin-releasing hormone
GOAT: Ghrelin O-acyl transferase
HIIT: High-intensity interval training
HPG: Hypothalamic-pituitary-gonadal
HR: Heart rate
kcal: kilocalorie
LH: Luteinizing hormone
LP: Luteal phase
MICT: Moderate-intensity continuous training
NPY: Neuropeptide Y
OP: Ovulatory phase
P_4: Progesterone
POMC: Pro-opiomelanocortin
PP: Pancreatic polypeptide
PVN: Paraventricular nucleus
PYY/$PYY_{3\text{-}36}$: Peptide tyrosine-tyrosine
SIT: Sprint interval training
VAS: Visual analogue scale
$\dot{V}CO_2$: Carbon dioxide production
VICT: Vigorous-intensity continuous training
$\dot{V}O_{2max}$: Maximal oxygen uptake
$\dot{V}O_{2peak}$: Peak oxygen uptake

CHAPTER 1:
LITERATURE REVIEW

Introduction

The prevalence of overweight and obesity in Canada has increased significantly over recent decades, with 36.3% of Canadians adults reporting as overweight, and 26.8% reporting as obese in 2018 (Statistics Canada, 2019). Sedentary lifestyles coupled with an excessive consumption of calorie-dense foods are important contributors to the current obesity epidemic, highlighting the preventable nature of this major public health concern. Ultimately, prevention of overweight and obesity centers on maintaining the body in a state of energy balance, when energy intake and energy expenditure are at equilibrium (Donnelly et al. 2005), resulting in a stable body mass over time. Increases in fat mass occur when energy intake continuously exceeds energy expenditure, whereas decreases in fat mass require a prolonged energy deficit, where energy expenditure continuously exceeds energy intake (Hall et al. 2012). Maintaining an energy deficit can be attained through increasing energy expenditure via exercise or spontaneous physical activity, decreasing energy intake by modifying diet, or both. However, in a society where sugar-laden, energy-dense convenience foods are readily available, maintaining energy balance through diet alone is proven difficult and may not provide a viable long-term solution as weight re-gain following dietary restriction has been demonstrated in more than 50% of individuals who undertake this method (Donnelly et al. 2005). Furthermore, dietary restriction has been shown to diminish resting metabolic rate and spontaneous physical activity, partially or entirely negating the intended energy deficit for weight loss. Thus, modifying only one component of energy balance appears to be inefficient for sustained weight management, and more effective interventions are required.

Exercise offers an alternative mechanism to restriction of food intake alone. As a widely-accepted method to improve cardiorespiratory capacity, the health-related benefits of exercise are

strongly supported (Astorino et al. 2012; Burgomaster et al. 2008; Burgomaster et al. 2005; Garber et al. 2011; Gibala et al. 2006; Gillen et al. 2014; Hazell et al. 2010; MacPherson et al. 2011; Whyte et al. 2010). The role of exercise in energy balance is also established, as it is well known that increasing energy expenditure with an acute bout of exercise can induce an energy deficit (Donnelly and Smith 2005). Indeed, exercise is frequently recommended as a mechanism to induce weight loss by many public health organizations (Brauer et al. 2015; Shephard et al. 2004). However, the energy deficit created through exercise does not always translate to weight loss (Donnelly et al. 2003). When performed at the recommended intensity and duration prescribed by the Canadian Society for Exercise Physiology (CSEP; ≥150 min/wk at a moderate intensity), weight loss is often less than anticipated (Thorogood et al. 2011), and the clinical effectiveness of exercise as a weight loss strategy has been questioned (Doucet et al. 2011; Foright et al. 2018; Thorogood et al. 2011).

Unfortunately, this finding is even more apparent in females, as though many exercise interventions are effective for improving body composition in men, the same interventions are often less effective in women (Anderson et al. 2007; Donnelly et al. 2003; Donnelly and Smith 2005; Hill et al. 1987; Potteiger et al. 2003; Sartorio et al. 2005; Westerterp et al. 1992). While there are few studies that compare fat loss in men versus women, evidence for a sex difference arises from studies demonstrating that men experience a greater magnitude of fat loss compared to females, who either lose a lesser magnitude or maintain weight, despite engaging in the same exercise protocol (Bagley et al. 2016; Ballor et al. 1991; Després et al. 1984; Donnelly et al. 2003; Donnelly and Smith 2005; Westerterp et al. 1992). The most convincing evidence stems from the first long-term, randomized, supervised, and sufficiently powered study (The Midwest Exercise Trial) conducted to compare weight losses between sexes due to exercise alone over a 16-month

period (Donnelly et al. 2003). Participants received identical exercise prescriptions based on intensity, frequency, and duration, progressing from a workload of 55% to 70% of maximal oxygen consumption ($\dot{V}O_{2max}$) for 20 to 45 minutes per session over the course of the study. Energy expenditure was assessed every 4 months using doubly labeled water and resting metabolic rate from indirect calorimetry in order to verify the minimum target of 400 kilocalories (kcal) per session. Furthermore, all sessions were performed under the direct supervision of research personnel to ensure the proper completion of prescribed exercise. The average energy expenditure per session equated to ~667 kcal and ~438 kcal for men and women, respectively, resulting in a mean difference of ~229 kcal per session, and after 16 months it was found that the women in the group maintained their previous weight (+0.6 kg of body mass), demonstrating no significant reductions in fat mass, whereas men lost ~5.2 kg of body mass, the majority of which (~96%) resulting from a significant loss in fat mass (~4.9 kg). Taken together, these findings demonstrate that while exercise can be used as an effective weight management tool in the absence of dietary restriction, women respond less favourably to exercise in terms of fat loss when exercise is prescribed as a relative intensity (Donnelly et al. 2003).

Others have argued that when energy expenditure is standardized at an absolute value for men and women (ex., 500 kcal), the sexually divergent body composition response is eliminated (Caudwell et al. 2013; Donnelly et al. 2013; Hagobian et al. 2013a; Schubert et al. 2013). This finding was demonstrated most convincingly in a second randomized, controlled, sufficiently powered and supervised study (The Midwest Exercise Trial 2) conducted by the same group, assessing weight loss between sexes in response to exercise eliciting the same energy expenditure (Donnelly et al. 2013; Donnelly et al. 2012). Participants were randomly assigned to expend either 400 or 600 kcal per session, 5 d/wk over a 10-month period, or to a no-exercise control group. The

same mechanisms were used to verify exercise completion as described above. Results demonstrated no significant differences in weight loss between men and women performing exercise equating to either 400 or 600 kcal per session, and it was concluded that exercise prescription using energy expenditure rather than intensity, frequency, and duration results in similar weight loss across the sexes (Donnelly et al. 2013). However, it should be noted that to achieve the same energy expenditure in an exercise session, women are required to work for significantly longer periods of time at the same intensity (Caudwell et al. 2013; Donnelly et al. 2013). In the above case, women required ~48 min to expend 400 kcal, whereas men required ~31 min, equating to a 17 min difference between the groups that accounted for 35% of the total session for women. Similarly, women required ~63 min/session to expend 600 kcal, compared to males who required ~42 min/session, resulting in a 21-min difference, accounting for 33% of the total session for women (Donnelly et al. 2013). These stark differences in the work requirement for males and females to achieve a similar level of fat loss suggest the existence of a sex difference, rather than demonstrate a lack thereof, as many researchers have purported. If men exercising for the same duration at the same relative intensity as women demonstrate a greater magnitude of fat loss, it is highly plausible to suggest that there may be an underlying factor affecting energy balance in females, whereby the exercise-induced energy deficit is partially or fully negated. The lower levels of fat loss in women exercising at the same relative intensity as men in combination with the discrepant work necessary to achieve an absolute level of energy expenditure, and thus similar fat losses, provide a strong argument for such an adaptive mechanism, and may underlie the demonstrated differences between men and women.

In general, the difficulties experienced with weight loss accompanying exercise are frequently attributed to adaptive mechanisms that some individuals recruit in response to exercise

(Donnelly and Smith 2005). These compensatory behaviours, such a decrease in spontaneous physical activity or an increase in energy intake (King et al. 2007), can oppose an energy deficit induced by exercise, ultimately minimizing or altogether negating the negative energy balance required for weight loss (King et al. 2012; Stubbs et al. 2004; Westerterp et al. 1992). Numerous studies have documented no compensatory increase in energy intake immediately to 8 days following an acute session of exercise in men (Hagobian et al. 2013b; King et al. 1997; Stubbs et al. 2002a), or women during the follicular phase of the menstrual cycle (Hagobian et al. 2013b; McLaughlin et al. 2006). However, partial compensation accounting for ~30% of the exercise-induced energy deficit has been documented when energy intake is tracked for a period of two weeks (Stubbs et al. 2004; Stubbs et al. 2002b; Westerterp et al. 1992; Whybrow et al. 2008). While this partial compensation response has been identified in both sexes, it has been suggested that women demonstrate greater adaptive responses as a mechanism to preserve reproductive functions, and more accurately match energy intake with energy expenditure to defend against weight loss (Hagobian et al. 2009; Wade et al. 2004). As such, it is possible that these differences in compensatory responses to exercise may partially explain the greater fat losses seen in men versus women.

In line with the notion that females may exhibit more potent compensatory responses to energy deficits in order to preserve reproductive function, it is plausible that the reduced efficacy of exercise for weight loss in females may be related to 17β-Estradiol (E_2) and progesterone (P_4), two of the main ovarian sex hormones involved in the menstrual cycle. These hormones are known to influence energy intake over the course of the cycle such that the fluctuations in energy intake coincide with fluctuations in sex hormones, suggesting a potential role in compensatory eating

(Dye et al. 1997; Johnson et al. 1994; Shi et al. 2009; Sinchak et al. 2012). However, before these changes are discussed, the physiology of a typical menstrual cycle will be briefly reviewed.

The Menstrual Cycle

Basic Physiology

In humans, ovulation occurs in spontaneous cycles that occur regularly from puberty to menopause and is known as the menstrual cycle. The menstrual cycle is under control of the hypothalamic-pituitary-gonadal axis (HPG axis), and involves a complex interaction between several hormones, including the hypothalamic neurohormone gonadotropin-releasing hormone (GnRH), pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as the gonadal steroid hormones E_2 and P_4 (Asarian et al. 2013; Bonen et al. 1983). Various forms of estrogen exist, but the most biologically active form in healthy young women is 17β-Estradiol (E_2) (Hirschberg 2012). GnRH is secreted from the hypothalamus, leading to subsequent secretion of the gonadotropins, FSH and LH, from the anterior pituitary. These hormones, in combination with E_2 and P_4, provide positive and negative feedback signals to the pituitary and hypothalamus that lead to changing hormone secretion throughout the menstrual cycle (Reilly 2000). Based on the different hormonal profiles, the menstrual cycle can be divided into distinct phases, including the follicular phase (FP), the ovulatory phase (OP), and the luteal phase (LP).

The FP begins with the onset of menses and is characterized by the development of the immature follicle into a secondary, mature follicle containing a mature oocyte. Throughout the FP, the developing follicle begins to release E_2, triggering a sharp rise in the secretion of LH to induce ovulation, or the release of the mature oocyte from the follicle. On average, ovulation

occurs ~36 h after the LH surge, on day 14 of the menstrual cycle, and constitutes the OP (Asarian and Geary 2013; Buffenstein et al. 1995). The cycle now enters the LP, in which the remaining portion of the secondary follicle develops into the corpus luteum and produces large amounts of P_4. The LP is thus characterized by a P_4-dominant state and terminates at the start of menses (Frankovich et al. 2000). Of the sex hormones involved in the menstrual cycle, both E_2 and P_4 have been shown to have important implications in the regulation of appetite (Hirschberg 2012).

The Menstrual Cycle and Energy Balance

A close coupling between the fluctuations of ovarian sex hormones throughout the phases of the menstrual cycle and energy intake has been well documented in both rats (Asarian and Geary 2002; Asarian and Geary 2006; Bartness and Waldbillig 1984;) and humans (Asarian et al. 2006; Asarian and Geary 2013; Bisdee et al. 1989; Buffenstein et al. 1995; Dye and Blundell 1997; Hirschberg 2012) (Figure 1). Specifically in humans, a plethora of studies demonstrate a decrease in energy intake throughout the FP that reaches a minimum during the OP when E_2 levels are greatest (Asarian and Geary 2013; Bisdee et al. 1989; Buffenstein et al. 1995; Dye and Blundell 1997; Hirschberg 2012). Conversely, the greatest energy intake is observed in the LP when P_4 is at its greatest (Brennan et al. 2009; Bryant et al. 2006; Buffenstein et al. 1995; Cross et al. 2001; Dye and Blundell 1997; Reed et al. 2008), with the average differences in energy intake between the OP and LP equating to ~275 kcal/day, and between the mid-FP and mid-LP equating to ~228 kcal/day (Asarian and Geary, 2013). These findings are highly reproducible and are consistently demonstrated despite considerable methodological variations between studies (Buffenstein et al. 1995). Interestingly, these cyclic fluctuations in eating do not occur during anovulatory cycles (Buffenstein et al. 1995), evidence which – collectively with the similar cyclicity of eating in rats

– suggest that eating behaviour is under the control of the HPG axis and influenced by ovarian hormones in females (Asarian and Geary 2013).

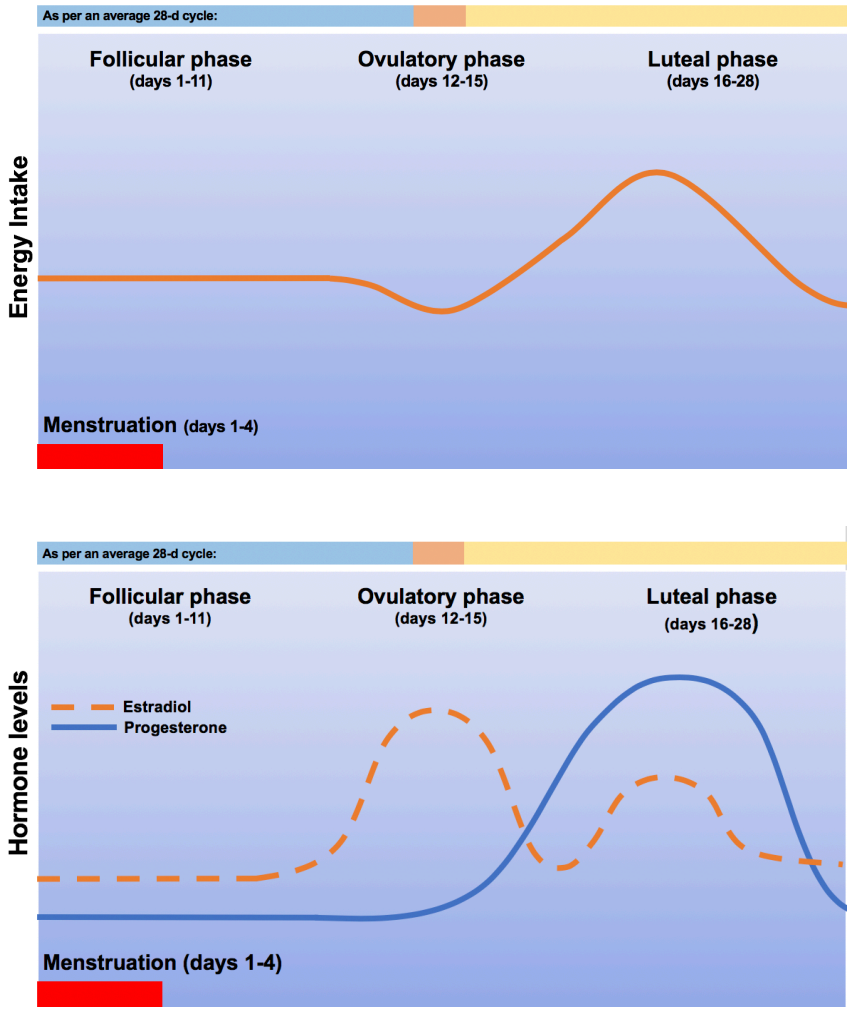

Figure 1: Schematic changes in energy intake (top) and gonadal hormones (bottom) across an average 28-day menstrual cycle. Energy intake is reduced in the periovulatory period when plasma estradiol (E_2) peaks, and energy intake increases in the luteal phase when plasma progesterone (P_4) is elevated [Adapted from Hirschberg 2012].

The effects of ovarian hormones on eating behaviour has been studied in detail with rodent models and it is hypothesized that the reduced energy intake in the OP may be attributed to an appetite-inhibiting, or anorexigenic effect of E_2 (Sinchak and Wagner 2012; Wade 1972). Ovariectomy of adult female rats (thus with a substantial decrease in circulating E_2) demonstrate

an immediate and sustained increase in energy intake, weight gain, and adiposity (Butera 2010; Kakolewski et al. 1968; Wade 1972). Reversal of these effects is observed when normal physiological levels of E_2 are restored (Asarian et al. 2002; Butera 2010; Wade 1972). Similarly, when E_2 is administered to the paraventricular nucleus of the hypothalamus (Butera et al. 1996) or the ventromedial hypothalamus (Wade and Zucker 1970) of ovariectomized rats, a decrease in energy intake is observed, whereas direct placement of E_2 in brain regions not involved in the control of feeding behaviour do not show this response (Butera et al. 1996).

The effects of P_4 on eating behaviour are less clear, as it is always present with increasing concentrations of E_2, and no methods exist to isolate its effect across the menstrual cycle (Stachenfeld et al. 2014). Despite this methodological shortcoming in humans, the impact of ovariectomy and hormone therapy in rodent models suggests P_4 does not have a direct orexigenic effect, as treatment with P_4 does not have a significant influence on energy intake unless administered in abnormal, supra-physiological doses (Butera 2010). Thus, these findings suggest P_4 is not directly involved in altering feeding behaviour, and rather than exerting an orexigenic effect, an anti-estrogenic effect appears to be more plausible (Frankovich and Lebrun 2000; Wade 1972). Certain metabolic actions of E_2 are antagonized in the presence of P_4 (Frankovich and Lebrun 2000), and it is possible that the appetite-inhibitory effects of E_2 are also antagonized (Davidsen et al. 2007; Wade 1972). For example, when P_4 levels were artificially elevated in rhesus monkeys, E_2 levels were decreased, and the E_2 surge that precedes the LH surge did not occur (Hess et al. 1973). Taken together, these findings suggest that the increase in energy intake observed in the LP, characterized by its P_4-dominated state, may be partially attributable to a potentially anti-estrogenic effect of P_4.

It is important to discuss both components of energy balance (i.e., expenditure and intake) to provide a more holistic understanding of the potential effects of the menstrual cycle on weight management. If women are to maintain energy balance across the cycle, shifts in energy intake should be met with shifts in energy expenditure of the same magnitude. As such, it has been suggested that changes in resting metabolic rate (RMR) may mirror, or compensate for, the demonstrated fluctuations in energy intake (Webb 1988), and that increases in total daily energy expenditure may be due to cyclic changes in RMR, the largest portion of daily energy turnover. Similar fluctuations in 24-h energy expenditure have been reported between phases, equating to an increase of ~89-279 kcal/day, or 2.5-11% in the LP vs. the FP (Davidsen et al. 2007), though are less consistent than the changes observed with energy intake (Buffenstein et al. 1995; Davidsen et al. 2007). Data from the same group of women are required to directly investigate the hypothesis of parallel changes between energy intake and RMR, and the only group to investigate this relationship demonstrated changes of similar magnitude (Pelkman et al. 2001). It is unknown whether the change in energy intake across the menstrual cycle is driven by the change in energy expenditure, or vice versa.

In absence of corresponding increases in energy expenditure, the consistently greater energy intake in the LP would lead to an energy imbalance and gradual increase in body mass over time, as chronic overconsumptions as small as ~50-100 kcal over time can result in obesity (Garland et al. 2011). A steady increase in body mass (primarily from an increase in fat mass) was exhibited in the control group of women over a period of 16 months during the Midwest Exercise Trial (Donnelly et al. 2003), whereas this weight gain was prevented in the group of exercising women over the course of study. It is possible that women in the control group did not correct for the energy intake imbalance between phases, as has been speculated to be the case for women with

a greater tendency towards obesity (Buffenstein et al. 1995). However, this speculation should be interpreted with caution, as a gradual increase in body mass was not observed over 10 months in the Midwest Exercise Trial 2 control group of women (Donnelly et al. 2013). In the former scenario, it is plausible to suggest that alterations in other components of energy balance (ex. spontaneous physical activity, and as discussed above, RMR) may occur that mimic the fluctuations in energy intake to maintain energy balance across the cycle. Such precise matching between intake and expenditure between phases could potentially explain the lack of weight change over time, and additionally may partially play a role in the sex differences in weight losses following exercise. Further work is required to more clearly elucidate the relationship between energy intake and energy expenditure across the menstrual cycle.

Despite the lack of consensus regarding the interplay between expenditure and intake across the menstrual cycle, evidence of a consistent imbalance of energy intake between the FP and LP remains strong, and is likely related to an anorexigenic, or appetite-inhibitory effect of E_2. The effects of E_2 on energy intake are linked to E_2 receptors in the hypothalamus, a key homeostatic brain region involved in appetite regulation (Butera 2010; Hirschberg 2012). Within the arcuate nucleus (ARC) of the hypothalamus exist two distinct neuronal populations: appetite-stimulating neurons that secrete neuropeptide Y (NPY) and agouti-related peptide (AgRP), and appetite-inhibiting neurons that secrete pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART). Among these neurons are numerous E_2 receptors, and as such it remains possible that E_2 exhibits its satiety-inducing effects via direct stimulation of these neurons (Butera 2010). Indeed, E_2 has been shown to stimulate POMC/CART activity, and inhibit NPY/AgRP activity in female rodents (Sotonyi et al. 2010). Taken together, the majority of evidence suggests E_2 as the primary link between the HPG axis and neural control of feeding (Asarian and Geary

2006; Asarian and Geary 2013; Davidsen 2007), implicating a strong inhibitory role on food intake. Importantly, the cyclic changes in eating, both in normally cycling rodents and in ovariectomized rats subject to E_2 treatment, is expressed as spontaneous changes in meal size rather than meal frequency (Asarian and Geary 2013). Though minimal, there is also evidence to indicate that cyclic fluctuations in eating in women are expressed as changes in spontaneous meal size rather than meal frequency (Brennan et al. 2009). This specificity provides valuable insight to the potential mechanisms underlying this process, and in light of the link between sex hormones and the neural control of feeding behaviour, it is conceivable that E_2, and to a lesser extent P_4, may exert their effects on energy intake through interactions with appetite hormones that regulate eating on a meal-to-meal basis.

Appetite Regulation

Overview

Human eating is a complex behaviour involving physiological, psychological, and neurological domains (Harrold et al. 2012). Key brain regions involved in energy balance, including the hypothalamus and brainstem, are central integrators of a network of endocrine signals from gut-derived hormones as well as neural signals transmitted from mechanoreceptors and chemoreceptors in the gut (Murphy et al. 2004). Both episodic (acute responses to food consumption) and tonic factors (chronic inputs reflective of total adiposity and energy stores) contribute to the control of feeding and together, these signals provide information to regulate energy intake (Harrold et al. 2012).

The specific hormones involved in altering perceptions of hunger and satiety are either orexigenic/appetite-stimulating or anorexigenic/appetite-inhibiting (Murphy and Bloom 2004). Peripheral signals converge on the brain and are integrated in the arcuate nucleus (ARC) of the hypothalamus, where specific neuronal populations modulate neuropeptide release. The orexigenic neurons express NPY and AgRP to promote increases in appetite whereas anorexigenic neurons express POMC and CART to promote decreases in appetite (Cone et al. 2001; Murphy and Bloom 2004). At the present, there is only one known orexigenic gut-derived hormone (ghrelin) though there are numerous anorexigenic hormones, including glucagon-like peptide 1 (GLP-1), peptide tyrosine-tyrosine (PYY), cholecystokinin (CKK), pancreatic polypeptide (PP), and amylin. However, as only ghrelin, GLP-1 and PYY are consistently demonstrated to respond to acute exercise and subsequently contribute to energy intake (Hazell et al. 2016; Schubert et al. 2014), only these hormones will be included in the scope of this review.

Gastrointestinal Hormones

Ghrelin – Ghrelin is a 28-amino acid peptide hormone released from specialized endocrine cells in the stomach during periods of energy restriction. It is actively converted to acylated ghrelin by the addition of an O-octanoyl group on serine 3 (an eight-carbon fatty acid) by the enzyme ghrelin O-acyl transferase (GOAT) in the stomach and small intestine (Yang et al. 2008). Although acylated ghrelin composes only ~10-20% of the total ghrelin (acylated and des-acylated), it is believed to be the only form that is involved in appetite-stimulation, and its acylation is a required step in order to exert its orexigenic effects (Ghigo et al. 2005). Once in its active (acylated) form, ghrelin crosses the blood-brain barrier and stimulates the NPY/AgRP neurons in the ARC region of the hypothalamus to increase appetite, and blocking NPY/AgRP signaling

diminishes this response (Kojima et al. 2005). Exogenous acylated ghrelin administration into the cerebral ventricles potently stimulates food consumption in both rodents (Kojima and Kangawa 2005) and humans (Wren et al. 2001). Plasma acylated ghrelin concentrations reflect episodic hunger signaling, with pre-prandial elevations rapidly diminished post-prandially in proportion to energy intake (i.e. caloric load), indicating its important role in meal initiation and termination.

Glucagon-like Peptide-1 (GLP-1) – GLP-1 is synthesized from a precursor protein (preproglucagon) and is secreted by L cells, a subpopulation of enteroendocrine cells located in the greatest density in the distal small intestine and colon (Lu et al. 2018). Two equipotent forms exist in circulation ($GLP\text{-}1_{7\text{-}37}$ and $GLP\text{-}1_{7\text{-}36}$), with $GLP\text{-}1_{7\text{-}36}$ representing the majority in circulating plasma (Drucker 2006; Orskov et al. 1994). Following a meal, GLP-1 is released into circulation and is rapidly degraded by dipeptidyl peptidase-4 (DPP-IV) to $GLP\text{-}1_{9\text{-}36}$. Reflective of its anorexigenic properties, direct administration of GLP-1 into rodent cerebrospinal fluid reduces energy intake (Tang-Christensen et al. 1996), and exogenous intravenous administration of GLP-1 inhibits energy intake in both lean and obese humans (Flint et al. 1998; Gutzwiller et al. 1999a; Gutzwiller et al. 1999b; Verdich et al. 2001).

Peptide YY (PYY) – PYY is co-secreted with GLP-1 from enteroendocrine L cells located in the small and large intestine and has two active forms: $PYY_{1\text{-}3}$ and $PYY_{3\text{-}36}$. Similar to GLP-1, $PYY_{1\text{-}36}$ is proteolyzed by the DPP-IV enzyme to $PYY_{3\text{-}36}$, which exerts more potent anorexigenic effects (Cummings et al. 2007) and is found in the greatest concentrations in circulation (Batterham et al. 2006). Reflective of its properties as an episodic, appetite-inhibiting signal, $PYY_{3\text{-}36}$ concentrations are elevated immediately following energy intake, and administration of $PYY_{3\text{-}36}$ inhibits energy intake in both rodents and humans (Batterham et al. 2003; Batterham et al. 2002).

Exercise Intensity and Appetite Regulating Hormones

Given the rising obesity epidemic and prescription of exercise for weight loss, there is a widespread interest in the effects of exercise on endocrine signals that ultimately impact energy balance. Acute exercise of varying protocols (Table 1) has demonstrated effects on plasma concentrations of appetite-regulating hormones, particularly acylated ghrelin, PYY, and GLP-1.

Table 1. Exercise characteristics (Gibala et al. 2006; Weston et al. 2014)

Type of training	**Protocol**
Moderate-intensity continuous training (MICT)	30-90 min; ~50-70% $\dot{V}O_{2max}$
High-intensity interval training (HIIT)	Repeated bouts of near maximal effort (i.e. ~80-100% % $\dot{V}O_{2max}$) separated with low activity or rest
Sprint interval training (SIT)	Potent form of interval training, traditionally involving four to six repeated, 30-s supramaximal (i.e. >100% $\dot{V}O_{2max}$) sprints separated with 4 min of rest or active recovery

Generally, research suggests that concentrations of acylated ghrelin, the only known orexigenic hormone, decrease after a bout of exercise while concentrations of the anorexigenic hormones PYY and GLP-1 increase (King et al. 2010a; Larson-Meyer et al. 2012; Schubert et al. 2014; Ueda et al. 2009a). Furthermore, some of these effects appear to occur in an intensity-dependent manner (Hazell et al. 2016; Islam et al. 2017). Much of the research examining the influence of acute exercise on appetite-regulating hormones has been conducted using moderate-intensity continuous training (MICT) protocols, however, more recent research has examined the impact of perturbations of greater intensity, including vigorous-intensity continuous training (VICT), high-intensity interval training (HIIT), and sprint interval training (SIT).

Ghrelin – Numerous studies have examined the impact of acute MICT on plasma acylated ghrelin concentrations and do not demonstrate consistent exercise effects. Some studies have shown suppressions ranging from 14-60% compared to pre-exercise or resting levels (Balaguera-

Cortes et al. 2011; Broom et al. 2009; Broom et al. 2017; Broom et al. 2007; Deighton et al. 2013a; Kawano et al. 2013; King et al. 2015; King et al. 2010a; King et al. 2011b; Martins et al. 2015; Marzullo et al. 2008; Shiiya et al. 2011; Tiryaki-Sonmez et al. 2013; Vatansever-Ozen et al. 2011; Wasse et al. 2013), others have not (Hagobian et al. 2013b; Islam et al. 2017; King et al. 2010b; King et al. 2011a; Larson-Meyer et al. 2012; Metcalfe et al. 2015; Sim et al. 2014), but a recent meta-analysis demonstrates an overall 16.5% suppression of acylated ghrelin following MICT (Schubert et al. 2014). In those studies where a reduction in plasma acylated ghrelin was observed, the greatest effects were seen with more strenuous protocols that used slightly higher intensities and longer durations of MICT (Broom et al. 2009; Deighton et al. 2013a; Islam et al. 2017; Shiiya et al. 2011). As such, an important role for exercise intensity has been hypothesized, whereby the suppressive effects are more pronounced following exercise of higher intensity (Hazell et al. 2016). Indeed, recent research using near-maximal and supramaximal intensities have elicited a more consistent decrease in plasma acylated ghrelin concentrations. Immediate and prolonged suppression occurred following a running bout of VICT (30 min at ~85% $\dot{V}O_{2max}$), and SIT (4 "all-out" efforts), while MICT (30 min at ~65% $\dot{V}O_{2max}$) was not different than control (Islam et al. 2017). Others have also reported acylated ghrelin suppressions following both HIIT (Martins et al. 2015) and various SIT protocols (Deighton et al. 2013a; Islam et al. 2017; Martins et al. 2015; Panissa et al. 2016; Sim et al. 2014). Notably, supramaximal exercise has elicited significantly greater and more prolonged suppressions than both MICT (Metcalfe et al. 2015; Sim et al. 2014) and VICT (Islam et al. 2017), supporting an overall intensity-dependent acylated ghrelin reduction in response to acute exercise.

GLP-1 – Much less research exists examining the effects of acute exercise on plasma GLP-1 concentrations, with majority using MICT as the primary stimulus. Compared to rest, increases

ranging from 11-50% (Islam et al. 2017; Ueda et al. 2009a; Ueda et al. 2009b) and 16-1477% (Hallworth et al. 2017; Hazell et al. 2017a; Larson-Meyer et al. 2012; Martins et al. 2007; Martins et al. 2015) have been observed in the concentrations of plasma active GLP-1 and total GLP-1, respectively, following 30-60 min bouts of MICT at 50 – 75% $\dot{V}O_{2max}$. A recent meta-analysis revealed an overall increase of 13% following MICT (Schubert et al. 2014). Fewer studies have examined GLP-1 responses to high-intensity exercise and have yielded conflicting results. Both total (Hazell et al. 2017a; Martins et al. 2015) and active GLP-1 (Hazell et al. 2017a; Islam et al. 2017) are elevated following various SIT protocols, though another group reported no change in active GLP-1 concentrations following traditional SIT (Beaulieu et al. 2015). It is possible that total GLP-1 may respond differently than active GLP-1, however more work investigating the discrepant responses is required. Taken together, it appears as though exercise intensity is less closely associated with GLP-1 release, as evidenced by a similar release of active GLP-1 following both MICT and VICT (Hazell et al. 2017a), and other factors such as reaching a threshold of energy expenditure may be more important (Hazell et al. 2016). Further work is required to examine the relationship between high-intensity protocols and GLP-1.

PYY – Several studies demonstrate increases in both total (Broom et al. 2009; Deighton et al. 2013a; Hallworth et al. 2017; Hazell et al. 2017b; Islam et al. 2017; Kawano et al. 2013; Martins et al. 2007; Ueda et al. 2009b) and active (Deighton et al. 2014; Deighton et al. 2013b; King et al. 2011a; Larson-Meyer et al. 2012; Russell et al. 2009) plasma PYY concentrations following 30-60 min bouts of MICT at 50-70% $\dot{V}$O2max, with an overall 9% increase demonstrated in a meta-analysis (Schubert et al. 2014). As with acylated ghrelin, more strenuous MICT protocols performed at higher intensities (≥ 70% $\dot{V}$O2max) appear to strengthen this response (Broom et al. 2009; King et al. 2011a; Larson-Meyer et al. 2012; Ueda et al. 2009a), which is in line with recent

findings demonstrating a greater increase in PYY following VICT versus MICT (Islam et al. 2017). In contrast, no changes were found following several HIIT protocols (Martins et al. 2015; Metcalfe et al. 2015; Sim et al. 2014) or a SIT protocol designed to induce a 250 kcal deficit, or a short-duration SIT protocol inducing a 125 kcal deficit (Martins et al. 2015). Additionally, there may be no difference between traditional SIT (Table 1) and MICT (Islam et al. 2017) though this could be a result of a lower exercise volume (4 bouts of SIT) compared to previous protocols using 6 "all-out" bouts (Hazell et al. 2017a). Ultimately, these conflicting results prevent an unequivocal conclusion to the effects of high-intensity intermittent exercise on PYY and suggest future research should include PYY.

Appetite Perceptions and Energy Intake

Intimately linked with the fluctuations in appetite-regulatory hormones post-exercise are changes in perceptions of appetite, the subjective measures of the motivation to eat (Gibbons et al. 2019). Appetite is measured via ratings of hunger, fullness, satisfaction, and prospective food consumption, including a combined score of each component to calculate overall appetite, and is typically assessed using a visual analogue scale (VAS; Gibbons et al. 2019). Appetite is influenced by acute energy deficits created through exercise, and the interactions between exercise and the appetite system have received considerable interest as a result of their direct implications for body weight regulation. Often, the post-exercise decreases in acylated ghrelin and increases in GLP-1 and PYY occur simultaneously with a transient suppression of overall appetite (Deighton and Stensel 2014; Dorling et al. 2018), and collectively contribute to an anorexigenic environment that may influence the impact of exercise on energy balance. However, while some groups have reported post-exercise reductions in free-living energy intake (Hagobian et al. 2013; Islam et al.

2017; Sim et al. 2014), the majority of current evidence highlighted in a recent meta-analysis demonstrates that single bouts of moderate- to high-intensity exercise do not alter free-living energy intake immediately post-exercise (Schubert et al. 2014), suggesting that acute appetite suppression does not necessarily translate into reduced energy intake. However, the results of the meta-analysis did not suggest that individuals compensated for the exercise-induced energy deficit with an increased energy intake post-exercise, thereby resulting in a decrease in *relative* energy expenditure, and highlighting the important role that exercise plays in energy balance and long-term weight management.

Sex Differences in Appetite-Regulation

A major issue in the majority of the appetite regulation research is a primary focus on males and far less research on females. This is likely due to difficulty in recruiting and collecting data on females due to the menstrual cycle, as related hormonal fluctuations across the menstrual phases are potential confounding factors. Thus, even when females are included as research subjects, they are often studied in the early FP of the menstrual cycle when E_2 and P_4 concentrations are minimal (Alajmi et al. 2016; Hagobian et al. 2009; Hagobian et al. 2013b; Hallworth et al. 2017; Hazell et al. 2017b; Larson-Meyer et al. 2012; Panissa et al. 2016). However, evaluating appetite regulation during a time that reflects only a portion of an adult female's life prevents a complete understanding of appetite regulation in females. This is extremely problematic due to the previously described issues with women and fat loss from exercise as it is plausible that exercise may affect appetite regulating hormones differently in females than males, thereby altering post-exercise appetite control and contributing to the apparent sex difference in intervention effectiveness. Thus, evaluation of the sex-specific hormone response to exercise is necessary.

In the limited studies that did distinguish between men and women, reports of a sex difference are present, though equivocal (Alajmi et al. 2016; Hagobian et al. 2009; Hagobian et al. 2013b; Hazell et al. 2017b; Panissa et al. 2016). MICT (50-70% $\dot{V}O_{2max}$) decreases acylated ghrelin in males (Alajmi et al. 2016; Broom et al. 2009; Islam et al. 2017; King et al. 2010a; Schubert et al. 2014) and females by ~22% (Alajmi et al. 2016; Howe et al. 2016), though acylated ghrelin levels were significantly increased by ~25% (Hagobian et al. 2009) and 55% (Larson-Meyer et al. 2012) in women following a bout of cycling and running MICT (50-70% $\dot{V}O_{2max}$), respectively, while there were no differences in men (Hagobian et al. 2009). These findings suggest that exercise alters acylated ghrelin to favour a post-exercise orexigenic environment in women only. However, in more recent work directly comparing the appetite-regulatory response in sexes, no differences in acylated ghrelin were found between men and women following running or cycling MICT performed at 60-70% $\dot{V}O_{2max}$ (Alajmi et al. 2016; Hagobian et al. 2013b; Panissa et al. 2016), SIT (60 x 8-s "all out" cycling sprints), or HIIT (1 min cycling repetitions at maximal aerobic power) (Panissa et al.2016). Interestingly, one group reported no differences between acylated ghrelin elevations following MICT and VICT in females, though both protocols significantly suppressed acylated ghrelin immediately post-exercise by ~22% (Howe et al. 2016).

As with acylated ghrelin, there are inconsistent results regarding a sex difference in PYY response. MICT (50-70% $\dot{V}O_{2max}$) increases PYY in males (Broom et al. 2007; Deighton et al. 2013b; Hazell et al. 2017a; Islam et al. 2017) and females by ~9.5-25.0% (Hagobian et al. 2013b; Hazell et al. 2017b; Howe et al. 2016; Larson-Meyer et al. 2012), though others reported no significant elevation in PYY following MICT in females (Alajmi et al. 2016; Hallworth et al. 2017). Some suggest the increase may be greater in females than males (Hagobian et al. 2013b), however others have demonstrated greater PYY in response to HIIT (60 x 8s sprints; (Panissa et

al. 2016) and both MICT (30 min at 65% $\dot{V}O_{2max}$) and SIT (6 x 30s "all out" sprints; (Hazell et al. 2017b) in males versus females. Furthermore, VICT significantly increased PYY in females (~9.5%), however there was no difference between VICT and MICT (Howe et al. 2016), further demonstrating the need for future research to uncover potential mechanisms involved in the effect of exercise on appetite regulation.

Lastly, even less research exists examining a sex difference in GLP-1 where MICT (50-70% $\dot{V}O_{2max}$) increases total and active GLP-1 immediately post-exercise in males (Islam et al. 2017) and females by ~6.6-77% (Hallworth et al. 2017; Hazell et al. 2017b; Howe et al. 2016; Larson-Meyer et al. 2012; Martins et al. 2007), however no change was observed following walking MICT in females (Larson-Meyer et al. 2012). As with PYY, VICT significantly increased GLP-1 in females (~77%), however no difference was observed between VICT and MICT (Howe et al. 2016). Interestingly, in the only study to directly compare the GLP-1 response between men and women, a clear sex difference was noted. GLP-1 was significantly elevated in women immediately post MICT and SIT (6.6-8.2%), while no change was observed in men (Hazell et al. 2017b). This is in contrast to previous research demonstrating consistent post-exercise GLP-1 increases in men, however further work is necessary to comment on a sex difference in GLP-1 response to exercise.

Again, it is important to note that each study examining sex differences measured appetite-regulatory responses to exercise in the FP of the menstrual cycle, minimizing the effects of the female sex hormones. At present, only three studies have examined appetite-regulatory hormones during the LP of the menstrual cycle (Brennan et al. 2009; Campolier et al. 2016; McKie et al. 2018), and none have done so with exercise, which may present different findings than those described above due to the characteristic fluctuations of E_2 and P_4 across the phases. Indeed, one

group demonstrated that E_2 and P_4 fluctuations were associated with distinct hormonal responses to feeding across the menstrual cycle at rest (McKie et al. 2018). The P_4-dominant LP was associated with greater fasting concentrations of acylated ghrelin, while E_2 was strongly correlated with fasting GLP-1 in the FP only. In addition, the post-prandial change in active PYY was lower during the LP compared to the FP. In light of these differences, it is plausible that female sex hormones may play a role in the appetite-regulatory response to exercise as they have been suggested to be involved in energy intake changes across the cycle. Thus, it is important to study females in other phases of the menstrual cycle, when a sex difference may be more likely to occur.

Sex Hormones and Exercise Intensity

At present, there exists minimal research exploring ovarian sex hormone responses to exercise intensity. In the few studies that are available, it is evident that acute exercise of moderate to vigorous-intensity is associated with increases in plasma concentrations of E_2 and P_4 similarly across the phases of the menstrual cycle (Table 2).

Consistently, exercise performed at a low intensity (≤40% $\dot{V}O_{2max}$) does not significantly increase plasma concentrations of E_2 and P_4 in either phase (Bonen et al. 1991; Jurkowski et al. 1978; Redman et al. 2003), though a single study has demonstrated a significant increase in P_4 with exercise at ~40% $\dot{V}O_{2max}$ during the LP (Jurkowski et al. 1978). In the FP, elevations in E_2 and P_4 during exercise are not typically observed, unless exercise is performed at vigorous (80-95% $\dot{V}O_{2max}$) intensities (Bonen et al. 1991; Jurkowski et al. 1978), though moderate intensity (~75% $\dot{V}O_{2max}$) exercise has also been shown to increase P_4 (Bonen et al. 1979). In the LP, both E_2 and P_4 are significantly elevated during moderate (~65-75% $\dot{V}O_{2max}$) and vigorous (~80-95%

Table 2. Sex hormone response to exercise intensity

Paper	Intensity	%Δ [Absolute Δ]			
		FP		LP	
		E_2	P_4	E_2	P_4
Jurkowski et al. 1978	Light ~40% VO_{2max}	6.26% [23.86]	7.10% [0.12]	9.32% [68.29]	13.90%* [4.01]
	Moderate ~60% VO_{2max}	15.22% [58.0]	41.42% [0.75]	36.59%* [267.99]	38.94%* [11.23]
	Heavy ~90% VO_{2max}	25.05%* [95.45]	63.91% [1.08]	35.39%* [259.18]	37.83%* [10.91]
Bonen et al. 1979#	Light				
	Moderate ~75% VO_{2max}	-26.34%^ [-50.66]	40.16%^* [0.51]	15.60%* [152.35]	21.61%* [1.56]
	Heavy				
Bonen et al. 1991#♦	Light ~40% VO_{2max}	22.22% [40.0]	18.75% [0.3]	37.14% [130.0]	6.89% [7.69]
	Moderate				
	Heavy ~80% VO_{2max}	38.89%* [70.0]	112.50%* [1.8]	80.0%* [280.0]	48.28%* [53.85]
Redman et al. 2003	Light ~25% VO_{2max}	40.79% [35.2]	8.0% [0.2]	-1.39% [-5.1]	6.25% [1.4]
	Moderate ~75% VO_{2max}	84.24% [72.7]	28.0% [0.7]	31.24%* [116.3]	15.63% [3.5]
	Heavy				

Note: All %Δ values were manually calculated.
%Δ = (post-exercise – pre-exercise)/pre-exercise x 100.
E_2 = pmol·L^{-1}; P_4 = nmol·L^{-1}; ^ = FP session occurred during menses. * = Significantly different from resting values. # = Data extrapolated from figures.
♦ = Researchers did not distinguish significance between exercise intensities.

$\dot{V}O_{2max}$) exercise (Bonen et al. 1991; Jurkowski et al. 1978) suggesting a dose-response effect that may affect P_4 more than E_2.

It is important to note that these elevations are observed during the exercise itself, as blood samples were obtained in the final minutes of work performed at each of the relative intensities (i.e. 40%, 80%, exhaustive exercise). Where post-exercise changes were sampled and reported, findings are equivocal. An elevation of P_4 in the FP persisted 30 min into the post-exercise period in one study (Bonen et al. 1991), however others report a fall in plasma concentrations of both E_2

and P_4 in the LP within this time frame (Bonen et al. 1991; Jurkowski et al. 1978). Additionally, all studies that examined the influence of exercise intensity on these hormones utilized one session per phase, whereby exercise at a low intensity was immediately followed by exercise at a higher intensity. A limitation of this design is the lack of ability to distinguish between an intensity or duration effect, as no study to date has examined the sex hormone response to intensity in separate sessions consisting of either moderate or vigorous intensity alone. Thus, it is unknown whether the significant elevations in E_2 and P_4 following MICT and VICT are due to the greater intensities of the exercise, or due to the long durations (>40 min) of exercise. Further work in this area is required to distinguish between these effects. An additional limitation is the timing of testing, as this was inconsistent between studies, occurring at different times throughout the menstrual cycle. The timing of testing may have important implications due to the characteristic fluctuations of ovarian sex hormones across the menstrual cycle. Whereas hormones concentrations are low in the FP, testing during the LP should occur mid-stage, as this is when P_4 concentrations are maximal.

Consequently, as a result of the demonstrated impact of exercise intensity on female sex hormones, whereby no elevations tend to occur in the FP, and elevations occur in the LP in a dose-response fashion, implementing a vigorous-intensity exercise protocol across the FP and LP may offer a unique paradigm to study the effects of sex hormones on appetite regulation.

Summary

Aerobic exercise frequently results in a greater magnitude of fat loss in males but is less effective in females. The difficulties females experience may be due fluctuations in sex hormones throughout the menstrual cycle, particularly E_2 and P_4, as these hormones have been linked to

differences in energy intake and may ultimately result in a compensatory response that negates the exercise-induced energy expenditure. As energy intake is partially controlled through physiologic mechanisms involving appetite-regulating hormones that are either orexigenic or anorexigenic, examining whether E_2 and P_4 are involved in appetite-regulation is warranted. Few studies to date have investigated females in this field of research, and all have done so exclusively in the FP of the menstrual cycle. However, examining the effect of elevated E_2 and P_4 during the LP on appetite-regulating hormones will offer new insights into potential mechanisms involved in appetite regulation, and may lead to improved strategies for weight loss for women.

Purpose

Thus, the purpose of this study was to utilize VICT as a method to modulate plasma concentrations of E_2 and P_4 and examine their associations with post-exercise alterations in acylated ghrelin, PYY_{3-36}, active GLP-1, subjective appetite perceptions, and free-living energy intake during the follicular and luteal phases of the menstrual cycle.

Hypotheses

1. In the FP, E_2 concentrations will be elevated in response to VICT, along with post-exercise elevations in active GLP-1, PYY_{3-36} and decreases in acylated ghrelin, favouring a suppression of overall appetite. No significant elevations in P_4 will be observed in the FP.
2. In the LP, both E_2 and P_4 concentrations will be elevated in response to VICT, along with post-exercise elevations in active GLP-1, PYY_{3-36} , and a suppression of acylated ghrelin.

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CHAPTER 2:
MANUSCRIPT

EXAMINING THE POTENTIAL INVOVLEMENT OF SEX HORMONES ON APPETITE REGULATION IN FEMALES ACROSS THE MENSTRUAL CYCLE

Introduction

In light of the current rates of overweight and obesity in Canada, an improved understanding of the impact of exercise on energy balance is required. Generally, it is known that increasing energy expenditure with an acute bout of aerobic exercise can induce an energy deficit (Donnelly et al. 2005). However, this deficit does not consistently translate to weight loss (Donnelly et al. 2003), and particularly not in females, who tend to exhibit lesser magnitudes of fat loss compared to males despite engaging in identical exercise protocols (Bagley et al. 2016; Ballor et al. 1991; Després et al. 1984; Donnelly et al. 2003; Donnelly and Smith 2005; Westerterp et al. 1992), or who require significantly extended durations of exercise at the same relative intensity to exhibit similar fat losses (Donnelly et al. 2013). The difficulties females experience with weight loss accompanying exercise may be due to fluctuations in sex hormones throughout the menstrual cycle, particularly estradiol (E_2) and progesterone (P_4), as these hormones have been linked to fluctuations in energy intake between phases (Asarian et al. 2013).

The menstrual cycle is under the control of the hypothalamic-pituitary-gonadal (HPG) axis and can be divided into three distinct phases, including the follicular phase (FP), the ovulatory phase (OP), and the luteal phase (LP). The FP begins with the onset of menses and is characterized by low levels of P_4, and low but increasing levels of E_2 that trigger a sharp rise in the secretion of luteinizing hormone (LH) to induce ovulation (Asarian and Geary 2013). Occurring ~36 hours following the LH surge on approximately day 14 of the cycle, ovulation constitutes the OP and initiates the change to the LP, which is characterized by a P_4-dominant state that terminates at the start of menses (Asarian and Geary 2013; Frankovich et al. 2000). Convincing evidence from studies in both animals (Czaja et al. 1975; Kemnitz et al. 1984) and humans (Brennan et al. 2009; Bryant et al. 2006; Buffenstein et al. 1995; Cross et al. 2001; Fong et al. 1993; Gong et al. 1989;

Johnson et al. 1994; Lissner et al. 1988; Lyons et al. 1989; Martini et al. 1994; Reed et al. 2008) suggest a link between these fluctuations in E_2 and P_4 with the changes in energy intake. Specifically, energy intake decreases throughout the FP to a minimum that coincides with the greatest levels of E_2 in the OP, and an increase in energy intake is observed in the LP when P_4 reaches its peak concentration. On average, the differences in energy intake between the OP and LP equate to ~275 kilocalories (kcal)/day, and that between the mid-follicular and mid-luteal phases equates to ~228 kcal/day (Asarian and Geary 2013). Interestingly, these cyclic fluctuations in energy intake are not present during anovulatory cycles (Barr et al. 1995; Rock et al. 1996). Taken together, it is plausible to speculate that cyclic changes in energy intake across the menstrual cycle are influenced by HPG axis function, suggesting the potential involvement of E_2 and P_4 in appetite regulation (Asarian et al. 2006; Asarian and Geary 2013; Hirschberg 2012).

The physiological control of energy intake, known as appetite regulation, involves a complex interplay between brain regions such as the hypothalamus and brainstem and the episodic and tonic signalling of gut-derived peptides (Hussain et al. 2013). On a meal-to-meal basis, energy intake is primarily mediated through episodic signalling, comprising of a number of peptides that are released from the gastrointestinal tract. Such peptides are classified as being either orexigenic (appetite-stimulating) or anorexigenic (appetite-inhibiting) in nature and influence our perceptions of hunger before a meal and post-prandial satiety to regulate acute energy intake (Murphy et al. 2006). Acylated ghrelin, the only known orexigenic hormone, is a 28-amino acid peptide released from specialized endocrine cells in the stomach that becomes biologically active (acylated) with the addition of an octanoyl group by the ghrelin O-acyl transferase (GOAT) enzyme (Yang et al. 2008). Glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY) are two of several known anorexigenic hormones and are both released from enteroendocrine L cells in the small and

large intestine (Murphy and Bloom 2006). These three peptides are notably perturbed by acute exercise, whereby acylated ghrelin is typically suppressed (Schubert et al. 2014), potentially to a greater extent following exercise of greater intensities (Islam et al. 2017), and is often accompanied by simultaneous increases in GLP-1 and PYY (Hazell et al. 2016; Schubert et al. 2014). These changes in appetite-regulatory hormones are accompanied by a transient suppression of appetite (Deighton et al. 2014; King et al. 2010; King et al. 1994), a phenomenon termed "exercise-induced anorexia" (Blundell et al. 2003), and is sometimes accompanied by post-exercise reductions in free-living energy intake, though this is inconsistent (Schubert et al. 2013).

The majority of current research on appetite regulation has been performed in lean, young, physically-active males, and while similar findings have been reproduced in the limited work involving females (Alajmi et al. 2016; Hagobian et al. 2013; Howe et al. 2016; Panissa et al. 2016), some sex differences have been identified (Hagobian et al. 2009; Hazell et al. 2017; Larson-Meyer et al. 2012). Importantly, these differences have been demonstrated in work exclusively examining females during the FP to control for the potential confounding effect of cycling E_2 and P_4 throughout the menstrual cycle (Alajmi et al. 2016; Hagobian et al. 2009; Hagobian et al. 2013; Hallworth et al. 2017; Hazell et al. 2017; Larson-Meyer et al. 2012; Panissa et al. 2016). However, the lack of research involving the LP prevents a complete understanding of appetite regulation in humans, and particularly for identifying sex-based differences, as it is plausible to suggest that other differences may emerge in the presence of a diverse hormonal environment. Only three groups have examined the appetite-regulatory response during the LP (Brennan et al. 2009; Campolier et al. 2016; McKie et al. 2018), and none have done so with exercise, which may present different findings than those described above due to the characteristic fluctuations of E_2 and P_4 across the phases. Thus, the purpose of the current study was to utilize vigorous intensity

continuous training (VICT), a mode of exercise demonstrated to increase plasma concentrations of sex hormones post-exercise, as a mechanism to modulate E_2 and P_4 in normally menstruating, premenopausal women and examine their associations with acylated ghrelin, PYY_{3-36}, active GLP-1, subjective appetite perceptions, and free-living energy-intake post-exercise in both the FP and LP of the menstrual cycle.

Methods

Participants

Sixteen females volunteered to participate in this study. Participants were eumenorrheic (having a regularly occurring menstrual cycle lasting within a range of 24-35 d, where cycle length within each participant varied by only 1-2 d) for a minimum of 1 y (Lebrun et al. 1995), and menstruating for a minimum of 3 y (Middleton et al. 2006). All participants were non-smokers, deemed "healthy" as per the CSEP Get Active Questionnaire (GAQ; Appendix A), and recreationally active, defined as no more than three exercise sessions per week and not involved in any structured training program within four months of study participation. Participants were excluded from participating in the study if they had been pregnant in the past 3 y or had plans to become pregnant during the time of study participation, used hormonal oral contraceptives within the past 6 months, or had a diagnosis of any eating disorders or metabolic diseases such as diabetes.

Participants provided a detailed history of their menstrual cycle for a minimum of the past 3 cycles. Day 1 of the menstrual cycle was defined as the onset of menses, and thereafter menstrual phase was subject-monitored through the use of ovulation kits to identify a surge in LH. Participants were provided with a urinary LH surge detection kit (Easy@Home, Easy Healthcare Corporation, IL, USA) and were provided with detailed instructions on when to begin testing (as

per manufacturer's instructions), how to use the kit, as well as a PDF of the user manual for reference. Menstrual phase was additionally monitored by oral body temperature upon awakening, where a sustained increase of ~0.3°C was indicative of ovulation (Forsyth et al. 2008). Temperature and LH testing outcomes were reported daily to the researcher via text/email, and phase was confirmed with subsequent hormonal testing of plasma E_2 and P_4 on study days. Prior to study initiation, experimental procedures were explained in detail and all participants provided written informed consent. Ethical approval was obtained from the Wilfrid Laurier University Ethics Committee for Research on Human Subjects in accordance with the 1964 Declaration of Helsinki.

Study Design

Participants completed two separate experimental sessions (~4 h each) in a randomized, counterbalanced order. Experimental sessions consisted of a running-based VICT protocol (80% $\dot{V}O_{2max}$) performed in each the FP and LP. Blood samples and subjective appetite perceptions were obtained at four time points during each experimental session at 0900 h (#1), 0950 h (#2), 1020 h (#3), and 1120 h (#4). Participants were instructed to record energy intake (quantity of food intake and beverage consumption) for a 3-day period in a provided food log, including the day prior to, the day of, and the day following testing. The dietary intake was then replicated in the 24 h prior to the next experimental session.

Pre-experimental Procedures

All participants completed one familiarization session prior to the first experimental session, in which they were screened for exclusion criteria and introduced to the study protocol

and equipment. Participants performed a graded exercise test to exhaustion on a motorized treadmill (4Front, Woodway, WI, USA) for the determination of $\dot{V}O_{2max}$. Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured continuously using an online breath-by-breath gas collection and analysis system (MAX II, Aenergy intake technologies, Pittsburgh, PA, USA). Prior to data collection the gas analyzer was calibrated with gases of known concentrations and with a 3-L syringe for flow. Each participant was fitted with a silicon facemask (7400 series Vmask, Hans Rudolph Inc. KS, USA) to ensure comfort and prevent leaking during gas measurements. Heart rate (HR) was recorded using an integrated HR monitor (FT1, Polar Electro, QC, Canada). Participants began the test with a standardized 5 min warm up (3.5 mph), after which they ran at a self-selected pace between 5-7 mph for the remainder of the test. Incremental increases in grade (2%) were applied every two min until volitional fatigue. After a 20-min rest period, a verification phase was performed to ensure confidence in the obtained $\dot{V}O_{2max}$ value. Participants ran at 110% of the maximal work rate obtained in the previous test until volitional fatigue. $\dot{V}O_{2max}$ was defined as the greatest 30 s average at which $\dot{V}O_2$ values plateaued despite increases in workload. Alternatively, two of the following criteria were met: 1) respiratory exchange ratio (RER) > 1.10, 2) maximal HR within ±10 bpm of age-predicted maximum (defined as 220 – age), or 3) voluntary exhaustion. Upon determination of $\dot{V}O_{2max}$, ~80% of this value was calculated as the target intensity during the VICT protocol. Following the $\dot{V}O_{2max}$ test, participants were acclimatized to the equipment as well as to the efforts required during the exercise protocol to reduce any learning effects in subsequent sessions.

Experimental Sessions

Prior to all experimental sessions, participants were asked to refrain from physical activity, alcohol, and caffeine for 12 h. Participants arrived at the laboratory at 0800 h after a 12 h overnight fast and remained in the laboratory for the following ~4 h (Figure 1). Upon arrival, a standardized breakfast was provided (Chocolate chip Clif bar, 29 $kJ \cdot kg^{-1}$ body weight), which participants were given 15 min to consume and 45 min to digest. Water was provided ad libitum throughout the session. Exercise commenced at 0910 h and consisted of a 5-min standardized warm-up (3.5 mph), a 30 min running-based VICT protocol, and a 5 min cool-down. Gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) was measured continuously throughout exercise with the gas collection system and HR monitor. Venous blood samples were obtained at four time points, including 0900 h (pre-exercise), 0950 h (immediately post-exercise), 1020 h (30 min post-exercise), and 1120 h (90 min post-exercise). Perceptions of appetite were assessed at the same blood-sampling time points using a visual analogue scale (VAS, Appendix B).

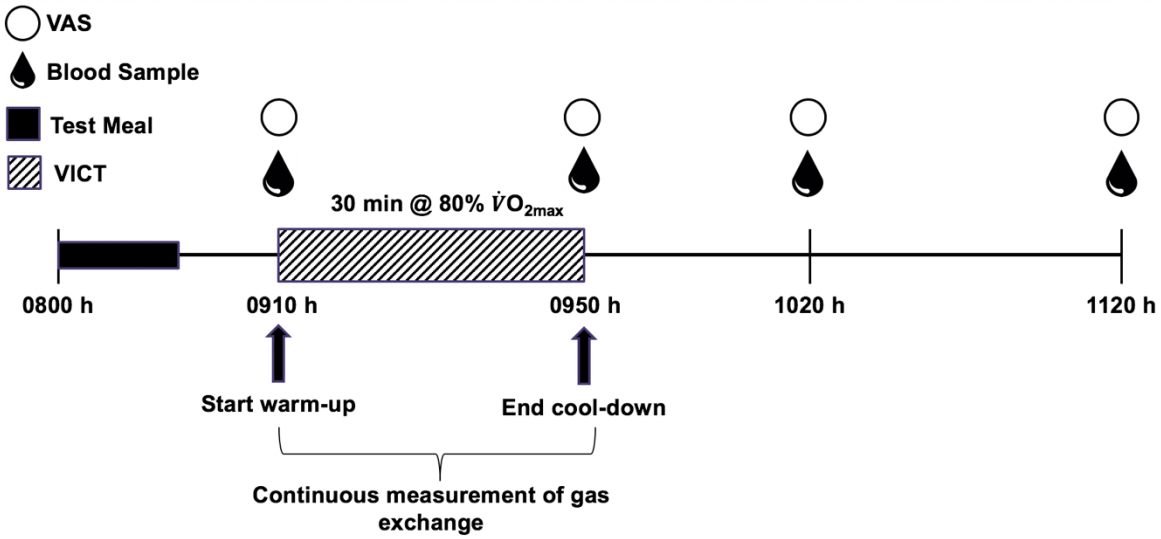

Figure 1: Visual representation of experimental session timeline. VAS = visual analogue scale.

Exercise Protocols

All exercise was performed on a motorized treadmill (4Front, Woodway, WI, USA) and consisted of 30 min of continuous running at a target workload of 80% $\dot{V}O_{2max}$. A pre-determined work rate was used at the start, calculated using the $\dot{V}O_2$ reserve method (Swain 2000). First, the target $\dot{V}O_2$ was determined by the following formula:

target $\dot{V}O_2$ = (intensity fraction)($\dot{V}O_{2max}$ - $\dot{V}O_2$rest) + $\dot{V}O_2$rest

Then, a mode-specific standardized equation (the ACSM running equation) was used to determine the percent grade necessary to elicit the target $\dot{V}O_2$ at each participants chosen speed (Swain 2000):

$\dot{V}O_2$ = 0.2 (speed) + 0.9 (speed)(grade) + 3.5

Thereafter, $\dot{V}O_2$ was continuously monitored, and work rate adjusted using speed and grade as needed to maintain the target intensity.

Blood Sampling Protocol

Venous blood samples were collected from an antecubital vein while participants were in a supine position for the determination of plasma acylated ghrelin, active GLP-1, PYY_{3-36}, E_2, and P_4. Two 3 mL whole blood samples were collected into separate pre-chilled Vacutainer tubes coated with K2 ethylenediaminetetraacetic acid (EDTA; 5.4 mg) at each time point. To prevent degradation of acylated ghrelin by proteolytic enzymes, 120 µL of 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) was added to whole blood immediately after sample collection in one tube. In the secondary tube, 30 µL of DPP-IV inhibitor was added to prevent inactivation of active $GLP\text{-}1_{7\text{-}36}$, $_{7\text{-}37}$ and ex-vivo conversion of $PYY_{1\text{-}36}$ to $PYY_{3\text{-}36}$, and 128 µL of aprotinin was added to prevent the degradation of $PYY_{3\text{-}36}$ by proteolytic

enzyme activity. E_2 and P_4 samples were analyzed from blood samples containing AEBSF (McKie et al. 2018). All tubes were gently inverted 10 times and centrifuged at 3000 g for 10 min at -4°C, after which the plasma supernatant was aliquoted into microcentrifuge tubes. Plasma from the acylated ghrelin vacutainer was acidified by the addition of 100 µL of HCl per 1 mL of plasma (Islam et al. 2017). All samples were stored at –80°C for subsequent analysis, whereby commercially available enzyme-linked immunosorbent assays (ELISA) were conducted to determine plasma concentrations of acylated ghrelin, active GLP-1, PYY_{3-36}, E_2, and P_4 as per manufacturer's instructions. All samples were analyzed in duplicate, except for a random 25% which were run in triplicate, and batch analyzed for each participant to eliminate inter-assay variation. The intra-assay coefficients of variation for E_2, P_4, acylated ghrelin, PYY_{3-36}, and active GLP-1 were 9.1, 10.5, 12.4, 8.4, 6.51, and 4.2%, respectively.

Appetite Perceptions

Appetite perceptions were assessed using a visual analog scale (VAS) that has been validated (Flint et al. 2000) and used in past research with both men (Islam et al. 2017) and women (Brennan et al. 2009). The scale consists of seven items and assesses participants' 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?"). Additional questions include: "How often do you eat breakfast in the morning?", "How anxious do you feel?", and "How nauseous do you feel?". Participants indicated their responses by reporting on a 100 mm scale with opposing statements (i.e. "not at all" and "extremely") at each end.

Energy Intake

Free-living energy intake was recorded for a 3-day period using self-reported dietary logs that were provided to each participant (Appendix C). On the day prior to an experimental session, participants were asked to begin recording their energy intake, and continue recording until the end of the day following the session. Participants were instructed to replicate their intake on the day prior to all subsequent sessions and were provided with a copy of their dietary log for that day. Detailed instructions were provided, including a sample log, to ensure accurate measurement and recording. Nutribase software (Nutribase Pro Edition, Cybersoft Inc., AZ, USA) was used to analyze all dietary logs for total energy intake (kcal) and macronutrient content each day.

Statistical Analysis

All data were analyzed using GraphPad PRISM (GraphPad Software, La Jolla, CA, USA). Paired samples t-tests were used to compare absolute pre-exercise acylated ghrelin, active GLP-1, PYY_{3-36}, E_2, and P_4 concentrations across the FP and LP, as well as absolute VAS scores at baseline and total energy intake (kcal) on the day prior to each experimental session. A series of two-factor (phase x time) repeated measures analyses of variance (RM ANOVAs) were conducted to compare changes in hormonal concentrations at each time point, as well as changes in appetite perceptions over time. Two-factor (phase x day) RM ANOVAs were used to compare changes in free-living energy intake on the day of and day after experimental sessions. Where significant main effects were found, post-hoc analyses using Tukey's HSD test were performed. Due to the individual variability of fasting absolute hormone concentrations between participants, changes at each time point for appetite-regulatory hormones were expressed as relative to each participants' pre-exercise values as described previously (Gibbons et al. 2013). Sex hormones are presented as

absolute values due to the expected differences between phases. The area under the curve (AUC) for blood-related parameters and appetite perceptions were calculated using the trapezoidal method, and all AUC values were compared via paired samples t-tests across the FP and LP. Pearson product-moment correlations were used to assess relationships between sex hormones and appetite regulatory hormones. Effect sizes were calculated using Cohen's d and Cohen's f for paired-samples t-tests and RM ANOVAs, respectively. Statistical significance was set at $P<0.05$, and all data are presented as mean±standard deviation.

Results

Participant Characteristics

A total of sixteen participants were recruited and successfully completed the familiarization session. However, one individual completed her first experimental session and then withdrew from the study, while four others withdrew after completing the familiarization session. Thus, eleven women successfully completed all experimental sessions. Due to time constraints, hormonal data presented are for eight participants, while the remaining appetite and energy intake data are for eleven. Participants were 22±4 years of age with a mean $\dot{V}O_{2max}$ of 37.02±4.04 $mL \cdot kg^{-1} \cdot min^{-1}$ (2.28±0.40 $L \cdot min^{-1}$) and the following physical characteristics: height 163.39±4.37 cm; weight: 61.95±10.60 kg; and BMI: 23.34±3.75 kg/m^2. Regarding the $\dot{V}O_{2max}$ test, 8 of 11 participants exhibited a plateau in $\dot{V}O_2$ uptake, defined as less than half of the expected increase in $\dot{V}O_2$ between subsequent stages (<1.35 $mL \cdot kg^{-1} \cdot min^{-1}$; Taylor et al. 1955). The remaining 3 reached 87.7% of the expected increase between the final two stages, however they satisfied at least two of the secondary criteria in the absence of a plateau. At the termination of their tests, these

participants had RER values of 1.17, 1.17 and 1.16, with heart rates of 200, 197, and 187 bpm, respectively. No verification phase exceeded the maximum value obtained on the $\dot{V}O_{2max}$ test. The mean $\dot{V}O_{2max}$ of the five individuals who withdrew from the study (38.58±3.63 $mL \cdot kg^{-1} \cdot min^{-1}$) was very similar to the participants who completed all experimental sessions, suggesting there was no difference in cardiorespiratory fitness between the two groups.

Exercise Responses

The 30-min VICT sessions in the FP and LP were completed at a work rate corresponding to 29.31±3.30 $mL \cdot kg^{-1} \cdot min^{-1}$ (79.3±0.03% $\dot{V}O_{2max}$) and 29.42±3.10 $mL \cdot kg^{-1} \cdot min^{-1}$ (79.6±0.02% $\dot{V}O_{2max}$), respectively, and were not significantly different between phases ($t_{(10)}$=0.324, P=0.753, *d*=-0.10). There were no significant differences in the average heart rate during VICT in the FP (171±15 bpm) and LP (171±12 bpm; $t_{(10)}$=0.127, P=0.902, *d*=-0.01), or in RER values during VICT in the FP (1.01±0.05) and the LP (1.00±0.04; $t_{(10)}$=2.13, P=0.058, *d*=0.64). $\dot{V}O_2$-derived estimates of energy expenditure (assuming a relationship of 5 kcal/L O_2) were 270.9±36.0 kcal and 272.5±45.5 kcal in the FP and LP, respectively, and were not significantly different between phases ($t_{(10)}$=0.662, P=0.523, *d*=-0.20).

Estradiol

All subjects reported regular menstrual cycles ranging 24-35 d in length. Average testing occurred on day 8±1.4 for the FP, and day 22±2.5 for the LP. There were no significant differences in the baseline concentrations of E_2 between the FP (52.72±12.79 $pg \cdot mL^{-1}$) and LP (76.82±28.46 $pg \cdot mL^{-1}$; $t_{(7)}$=1.99, P=0.086, *d*=-0.70). Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3,21)}$=0.44, P=0.727, *f*=0.25) for absolute changes in E_2 over

time (Figure 2). There was no main effect of phase ($F_{(1,7)}$=4.4, P=0.074, *f*=0.79), however there was a main effect of time ($F_{(3,21)}$=3.6, P=0.031, *f*=0.71), whereby E_2 was greater immediately post-exercise (76.72±34.15 pg·mL^{-1}) compared to pre-exercise (63.32±23.80 pg·mL^{-1}; P=0.041). AUC values were not different between phases (Figure 2; $t_{(7)}$=0.22. P=0.830, *d*=-0.08).

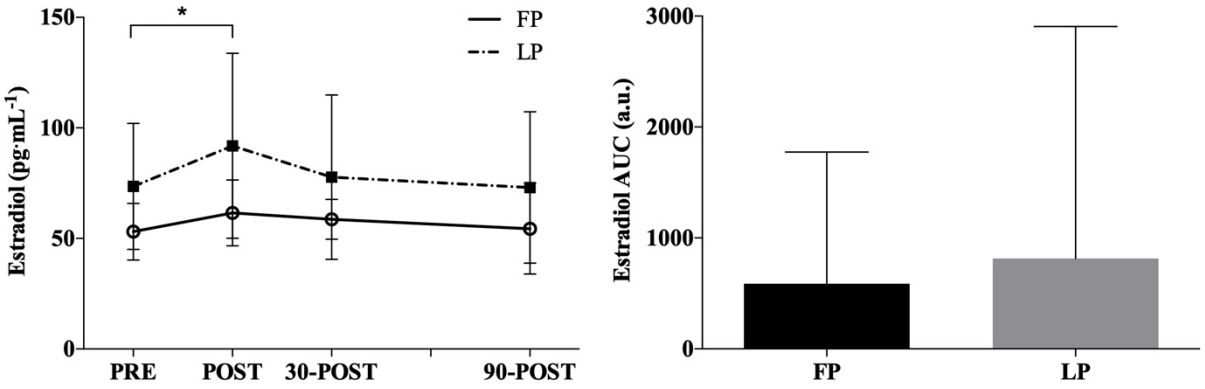

Figure 2: Absolute changes in E_2 across all time points (left) and area under the curve (AUC) values for E_2 (right). Note: FP: follicular phase; LP: luteal phase. * - POST significantly greater than PRE when collapsed across phases.

Progesterone

There were significant differences in the baseline concentrations of P_4 between phases, such that concentrations in the FP were significantly lower (3.07±1.76 ng·mL^{-1}) compared to the LP (12.28±6.14 ng·mL^{-1}; $t_{(7)}$=2.47, P=0.043, *d*=-0.87). Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3,21)}$=0.39, P=0.764, *f*=0.23) for absolute changes in P_4 over time (Figure 3), however, there was a main effect of both phase ($F_{(1,7)}$=9.9, P=0.016, *f*=1.19) and time ($F_{(3,21)}$=3.6, P=0.031, *f*=0.72). As expected, P_4 levels were significantly greater in the LP compared to the FP. Despite a significant main effect of time, no significant

differences were found following post-hoc analysis at any time point when phase was collapsed. AUC values were not different between phases (Figure 3; $t_{(7)}$=0.22. P=0.528, *d*=-0.23).

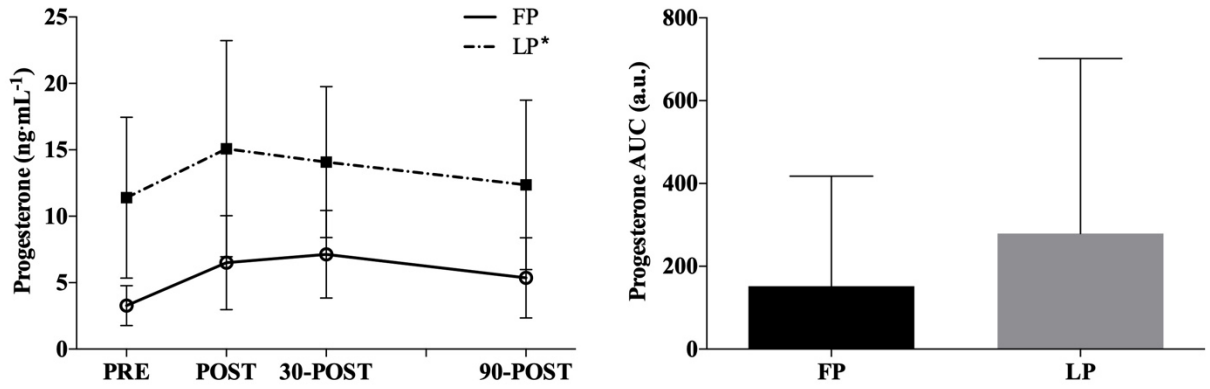

Figure 3: Absolute changes in P_4 across all time points (left) and area under the curve (AUC) values for P_4 (right). Note: FP: follicular phase; LP: luteal phase. * - significantly greater than FP.

Acylated Ghrelin

There was no significant difference in the baseline concentrations of acylated ghrelin across the FP and LP ($t_{(7)}$=1.92, P=0.097, *d*=-0.68). Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3, 21)}$=1.2, P=0.339, *f*=0.41) for changes in acylated ghrelin relative to baseline (Figure 4). There was a main effect of phase ($F_{(1, 7)}$=7.5, P=0.029, *f*=1.03), such that acylated ghrelin concentrations were significantly lower in the FP (138.24±87.81 pg·mL^{-1}) compared to the LP (206.84±137.02 pg·mL^{-1}, P=0.002). In addition, there was a main effect of time ($F_{(3, 21)}$=5.4, P=0.007, *f*=0.87), whereby acylated ghrelin concentrations were significantly greater at 90 min post-exercise (243.27±175.24 pg·mL^{-1}) compared to both immediately post- (136.96±86.80 pg·mL^{-1}; P=0.036) and 30 min post-exercise (136.92±86.70 pg·mL^{-1}; P=0.014). AUC values for acylated ghrelin (Figure 4) were not different between phases ($t_{(7)}$=1.15, P=0.289, *d*=-0.41).

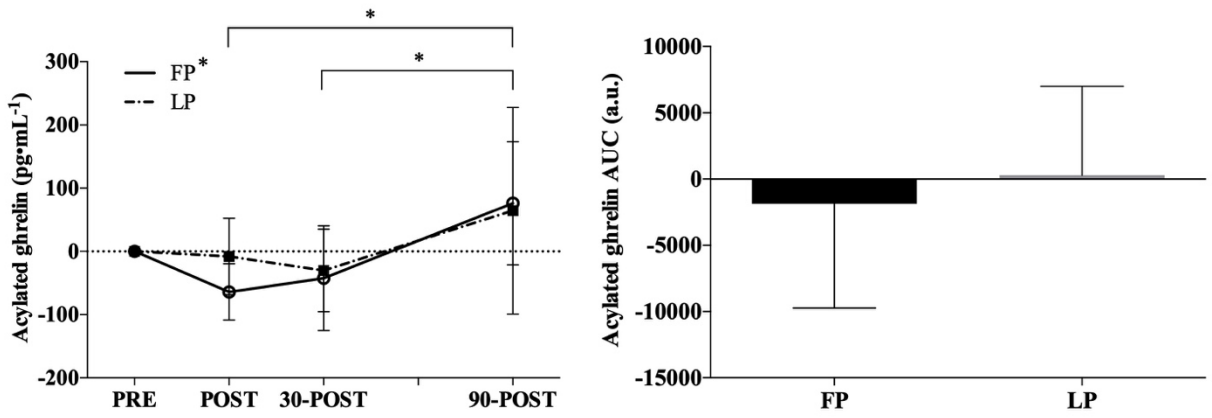

Figure 4: Changes in acylated ghrelin across all time points relative to baseline (left) and area under the curve (AUC) values for acylated ghrelin (right). *Note:* FP: follicular phase; LP: luteal phase; FP* – significantly different from LP; * - 90-POST significantly greater than POST and 30-POST when collapsed across phases.

PYY_{3-36}

There was no significant difference in the baseline concentrations of PYY_{3-36} across the FP and LP ($t_{(7)}$=0.47, P=0.652, *d*=-0.16). Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3, 21)}$=0.12, P=0.948, *f*=0.13) for changes in PYY_{3-36} relative to baseline (Figure 5). There was no main effect of phase ($F_{(1, 7)}$=0.001, P=0.973, *f*=0.18) or time ($F_{(3, 21)}$=1.3, P=0.305, *f*=0.41). AUC values for PYY_{3-36} (Figure 5) were not different between phases ($t_{(7)}$=0.10, P=0.926, *d*=0.04).

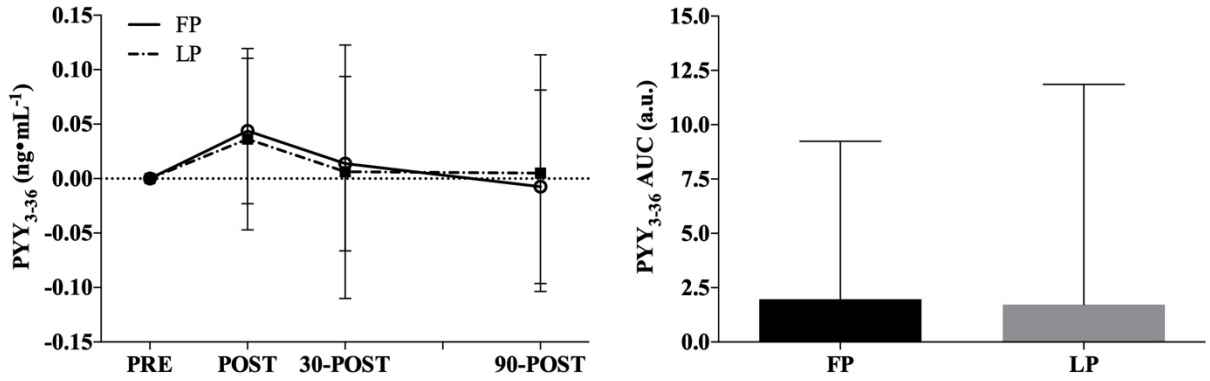

Figure 5: Changes in PYY_{3-36} across all time points relative to baseline (left) and area under the curve (AUC) values for PYY_{3-36} (right). *Note:* FP: follicular phase; LP: luteal phase.

Active GLP-1

There was no significant difference in the baseline concentrations of active GLP-1 across the FP and LP ($t_{(7)}$=0.47, P=0.654, *d*=0.17). Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3, 21)}$=1.55, P=0.238, *f*=0.47) for changes in active GLP-1 relative to baseline (Figure 6). There was no main effect of phase ($F_{(1, 7)}$=1.35, P=0.284, *f*=0.44), however there was a main effect of time ($F_{(3, 21)}$=3.8, P=0.026, *f*=0.73), whereby active GLP-1 concentrations were significantly elevated 30 min post-exercise (14.62±4.44 pM·mL^{-1}) compared to 90 min post-exercise (8.86±3.47 pM·mL^{-1}; P=0.016). AUC values for GLP-1 (Figure 6) were not different between phases ($t_{(7)}$=0.33, P=0.749, *d*=0.13).

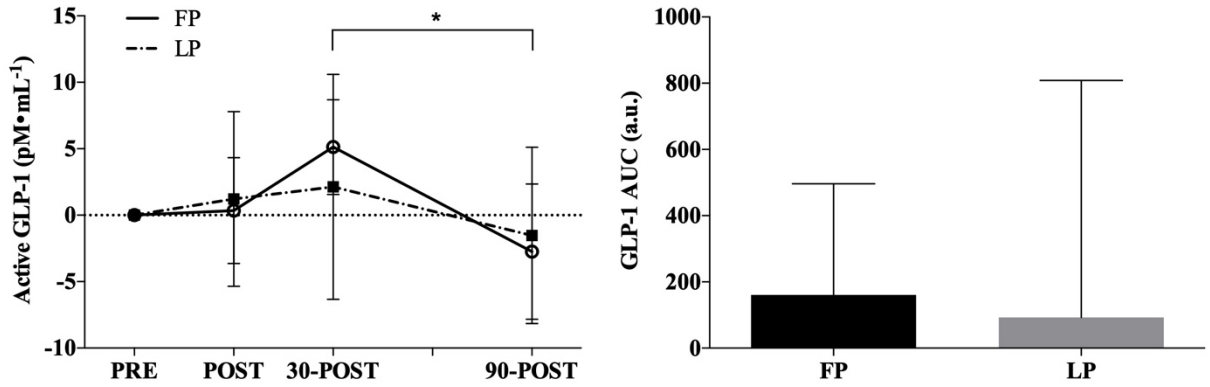

Figure 6: Changes in active GLP-1 across all time points relative to baseline (left) and area under the curve (AUC) values for GLP-1 (right). *Note:* FP: follicular phase; LP: luteal phase. * - 30-POST significantly greater than 90-POST when collapsed across phases.

Appetite Perceptions

There were no differences in absolute VAS scores pertaining to overall appetite ($t_{(10)}$=0.75, P=0.475, *d*=0.16) between the FP and LP at baseline. Two-factor (phase x time) repeated measures ANOVA revealed no significant interaction ($F_{(3, 30)}$=0.61, P=0.616, *f*=0.25) for changes in overall appetite relative to baseline (Figure 7). There was also no main effect of phase ($F_{(1, 10)}$=1.5, P=0.245, *f*=0.34), but there was a main effect of time ($F_{(3, 30)}$=14, P<0.001, *f*=1.17). Specifically, overall appetite was greater at 90 min post-exercise (66.19±19.59) compared to pre-exercise (41.95±21.63, P<0.001), immediately post-exercise (37.13±27.05, P<0.001), and 30-min post-exercise (50.81±25.09, P=0.023) when collapsed across phases. AUC values for overall appetite (Figure 7) were not different between phases (Figure 7; $t_{(10)}$=1.11, P=0.292, *d*=0.32). The results for each individual appetite perception can be found in Appendix D.

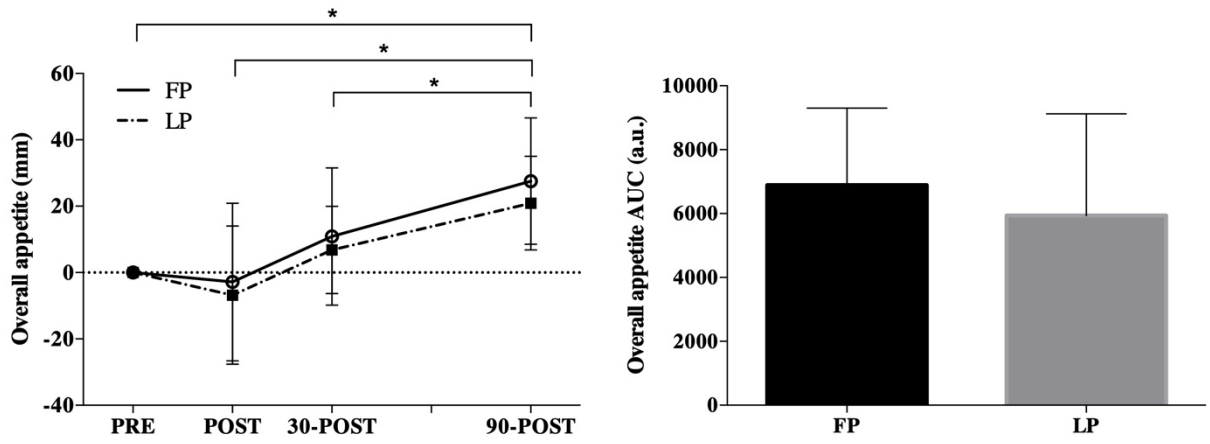

Figure 7: Changes in overall appetite across all time points relative to baseline (left) and area under the curve (AUC) values for overall appetite (right). *Note:* FP: follicular phase; LP: luteal phase. * - 90-POST significantly greater than PRE, POST, and 30-POST when collapsed across phases.

Energy Intake

There were no differences in energy intake between phases on the day prior to the experimental session ($t_{(9)}$=1.91, P=0.089, *d*=0.60), as was intended (Table 1). Two-factor (phase x day) repeated measures ANOVA revealed no significant interaction ($F_{(2, 18)}$=0.15, P=0.859, *f*=0.13) for changes in energy intake across the day before, day of, and day after experimental sessions (Figure 8). There was no main effect of phase ($F_{(1, 9)}$=0.54, P=0.481, *f*=0.24) or day ($F_{(2, 18)}$=1.5, P=0.244, *f*=0.40).

Table 1. Energy and macronutrient intake on the day prior to each experimental session.		
	FP	**LP**
Total energy intake (kcal)	1793.7±566.3	1757.7±553.3
Carbohydrate (g) (%)	204.8±96.5 54.2±0.2	202.5±95.2 54.4±0.1
Fat (g) (%)	75.2±28.1 21.9±0.1	74.1±25.4 21.9±0.1
Protein (g) (%)	78.9±30.8 24.0±0.1	76.50±27.4 23.7±0.1
Note: FP: follicular phase; LP: luteal phase		

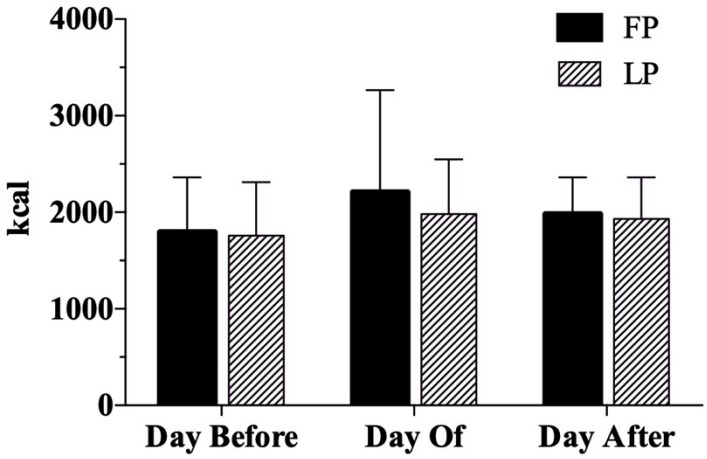

Figure 8: Total energy intake (kcal) on the day before, day of, and the day after each experimental session. *Note:* FP: follicular phase; LP: luteal phase.

Correlations

E_2 was positively correlated with acylated ghrelin at 90 min post-exercise in the FP (Table 2; r = 0.88, P=0.004), while P_4 was positively correlated with active GLP-1 at baseline in the FP (r = 0.78, P=0.022). There were no significant correlations at any other time point in either phase.

Table 2. Correlations between sex hormones and appetite-regulatory hormones.				
	E_2			
	PRE	**POST**	**30-POST**	**90-POST**
Acylated ghrelin				
FP	0.14	0.56	0.57	0.88*
LP	-0.02	-0.11	0.35	0.13
PYY_{3-36}				
FP	0.22	0.20	0.03	-0.19
LP	-0.42	-0.20	0.11	0.37
Active GLP-1				
FP	0.02	0.41	-0.38	-0.17
LP	-0.07	-0.16	-0.04	-0.17
	P_4			
	PRE	**POST**	**30-POST**	**90-POST**
Acylated ghrelin				
FP	-0.26	-0.32	-0.40	-0.03
LP	-0.06	-0.28	0.18	-0.02
PYY_{3-36}				
FP	0.01	0.26	0.23	-0.29
LP	-0.19	-0.12	0.20	0.30
Active GLP-1				
FP	0.78*	-0.06	0.47	0.21
LP	0.19	-0.56	0.35	-0.07
Note: Values are in Pearson's correlation coefficients (r). FP: follicular phase; LP: luteal phase *P<0.05				

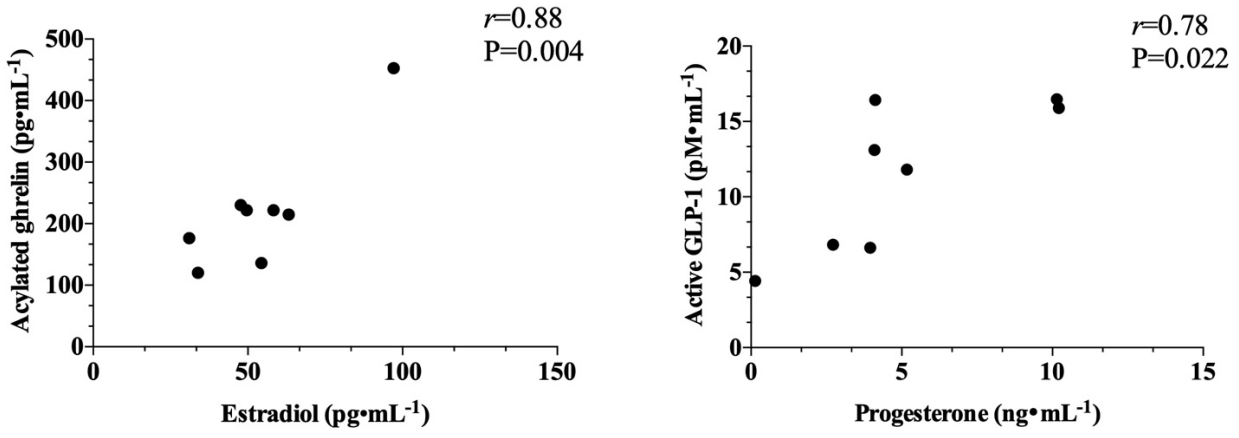

Figure 9: Pearson product-moment correlations (*r*) between E_2 and acylated ghrelin at 90 min post-exercise in the FP (left), and P_4 with active GLP-1 at baseline in the FP (right).

Discussion

The difficulties females experience with fat loss from exercise may be due to characteristic changes in E_2 and P_4 across the menstrual cycle, as these hormones coincide with changes in energy intake between phases and may be involved in the appetite-regulatory response to exercise. However, appetite regulation in females has been exclusively studied in the FP of the menstrual cycle where concentrations of E_2 and P_4 are low (Alajmi et al. 2016; Hagobian et al. 2009; Hagobian et al. 2013; Hallworth et al. 2017; Hazell et al. 2017; Larson-Meyer et al. 2012; Panissa et al. 2016), and the effects of greater concentrations of sex hormones in the LP on appetite-regulatory hormones is unknown. As such, the current investigation utilized vigorous running for 30 min at 80% $\dot{V}O_{2max}$ between the FP and LP in healthy, recreationally active, naturally menstruating pre-menopausal women to examine exercise-induced increases in E_2 and P_4 and their associations with alterations in appetite-regulatory hormones. The main findings of this study are: a) E_2 was significantly elevated immediately post-exercise, however P_4 was unaffected by VICT in both phases, b) acylated ghrelin was significantly lower in the FP compared to the LP, suggesting a selective post-exercise suppression in the FP only c) active GLP-1 concentrations were significantly elevated at 30 min post-exercise compared to 90 min post-exercise, and d) neither PYY_{3-36}, overall appetite, nor energy intake appeared to be affected by exercise or menstrual cycle phase.

Estradiol and Progesterone

As a major objective of this project was to observe the appetite-regulatory response to exercise in the LP, confidence that testing occurred in the correct phases is required. Though there were no significant differences in the baseline concentrations of E_2 between the FP (53.08±12.79

pg·mL^{-1}) and LP (73.57±28.46 pg·mL^{-1}), concentrations are in accordance with accepted values for each phase (Allen et al. 2016), and is similar to previous research where a significant difference between phases was not observed (Brennan et al. 2009; Reed et al. 2008). Baseline concentrations of P_4 in the FP are greater than the accepted values for this phase (Allen et al. 2016), suggesting that testing may have occurred in the mid- to late-FP, when concentrations of P_4 begin to rise, though they were significantly lower (3.07±1.76 ng·mL^{-1}) compared to the LP (12.28±6.14 ng·mL^{-1}). On average, experimental sessions during the FP occurred on day 8 of the cycle, with the latest day tested on day 10, as is common practice in other work (Brennan et al. 2009; Campolier et al. 2016; Hallworth et al. 2017). Plasma concentrations of P_4 in the LP are in accordance with the accepted values for this phase (Allen et al. 2016), and significant differences between phases are reflected in previous literature (Brennan et al. 2009).

Despite the baseline values and in accordance with our hypotheses, VICT elevated plasma concentrations of E_2 in both the FP and LP immediately post-exercise, demonstrating an increase of 15.97% and 24.90%, respectively, before declining close to baseline values at 30 min post-exercise. These magnitudes of change from baseline following vigorous-intensity exercise follow similar trends, albeit are slightly lower than that which has been presented in previous research at 25.05% and 35.39% in the FP and LP, respectively (Jurkowski et al. 1978), as are the concentrations we observed (Bonen et al. 1991; Jurkowski et al. 1978). Interestingly, our absolute changes are in line with those that occurred with light-intensity (Jurkowski et al. 1978) or moderate-intensity exercise (Redman et al. 2003) in both phases, as well as following moderate-intensity exercise in the FP (Jurkowski et al. 1978), though lesser changes in the LP. Despite these differences, our data appear to support the use of VICT as a mechanism to increase plasma concentrations of E_2 following exercise in each phase.

Contrary to our hypothesis, P_4 was not elevated in response to VICT in the LP. No significant differences were found following post-hoc analysis of P_4 (despite a main effect of time), though there was a trend for an increase in P_4 from pre- to post-exercise ($P=0.078$) with a medium effect size ($f=0.62$), suggesting an important increase may have occurred that appears to be driven by the post-exercise change in the LP compared to the FP (Figure 3). The magnitudes of change in P_4 observed following exercise in the FP and LP were 28.39% and 32.37%, respectively, and again, follow similar trends to previous research. The absolute changes we observed are greater than that which has been observed following light-intensity (Jurkowski et al. 1978; Redman et al. 2003) and moderate-intensity exercise (Bonen et al. 1979; Redman et al. 2003), and are in line with previous work following vigorous-intensity exercise (Jurkowski et al. 1978), though slightly lower than others (Bonen et al. 1991). Though similar to past work, it should be noted that absolute changes in P_4 were marginal in each phase, and the practical importance of such changes remains to be elucidated. A key methodological difference between our study and those aforementioned is the nature of the exercise bout, as exercise intensity gradually increased over time in previous work (Bonen et al. 1991; Bonen et al. 1979; Jurkowski et al. 1978; Redman et al. 2003), whereas our protocol involved a consistent intensity over 30 minutes. Thus, it is possible that the greater absolute changes observed in other work were due to a combination of an intensity and duration effect, and lesser magnitudes observed in our work are due to the shorter amount of time spent exercising. Future research should seek to distinguish between these effects, examining the ovarian hormonal response to acute sessions of exercise at various intensities.

Acylated Ghrelin

Contrary to our first hypothesis, we observed no significant suppression of acylated ghrelin immediately post-exercise in the FP (81.71±38.96 pg·mL^{-1}) compared to baseline (145.78±31.58 pg·mL^{-1}; P=0.157), though the effect size was large (*d*=1.53). However, together with the main effect of phase, whereby acylated ghrelin concentrations were significantly lower in the FP (138.24±87.81 pg·mL^{-1}) compared to the LP (206.84±137.02 pg·mL^{-1}), the 64.07 pg·mL^{-1} difference in plasma ghrelin concentrations from pre-post exercise in the FP compared to the 8.04 pg·mL^{-1} difference in the LP suggests a meaningful suppression occurred in the FP only. Few others have examined acylated ghrelin responses to exercise in women, and our observation is comparable to previous work in the FP demonstrating significant reductions in acylated ghrelin following 60 minutes of running at 70% $\dot{V}O_{2max}$ (Alajmi et al. 2016), HIIT performed at 100% $\dot{V}O_{2max}$ and "all-out" SIT (Panissa et al. 2016), and following isocaloric bouts of running performed at 60 and 85% $\dot{V}O_{2max}$ (Howe et al. 2016) during the FP in women. However, others have also reported increased (Hagobian et al. 2009; Larson-Meyer et al. 2012) or no change (Douglas et al. 2017; Hagobian et al. 2013) in acylated ghrelin following both running and cycling exercise performed at low (~50-65% $\dot{V}O_{2peak}$) to moderate (70-75% $\dot{V}O_{2peak}$) intensities, demonstrating a lack of consensus in the literature that is exclusively represented by research in the FP.

Similarly, in the LP, we observed no change in acylated ghrelin immediately post-exercise (192.22±87.45 pg·mL^{-1}) compared to baseline (200.26±100.35 pg·mL^{-1}; P=0.999, *d*=0.15), demonstrating no effect of VICT during the LP. This finding is in contrast to the majority of literature exhibiting a transient suppression of acylated ghrelin post-exercise (Schubert et al. 2014) and particularly following exercise of greater intensities, as acylated ghrelin has been shown to decrease in a dose-response fashion (Islam et al. 2017), though this trend is based on literature

performed in males only. As discussed above, the research in females is less consistent and there is no general consensus regarding the effect of acute exercise on acylated ghrelin specifically in women, highlighting the importance of studying appetite across the menstrual cycle. Our novel observation of acylated ghrelin in the LP compared to the response in the FP suggests that the appetite-suppressive effect of exercise may be blunted in the presence of greater concentrations of sex hormones during the LP. This finding is in line with the suggestion that women demonstrate greater adaptive responses to energy deficits as a mechanism to preserve reproductive functions, body fat stores, and energy balance (Hagobian et al. 2010; Hagobian et al. 2009; Wade et al. 2004), and particularly due to this finding occurring during the LP when the body is preparing to maintain pregnancy. Alternatively, production of acylated ghrelin in the FP may be impacted by E_2, as decreased numbers of ghrelin-producing cells, ghrelin mRNA concentrations, and plasma concentrations of ghrelin have been observed following exogenous E_2 administration in ovariectomized rats (Matsubara et al. 2004). This suggestion appears to be supported with our observed trend (P=0.097, *d*=-0.68) of higher baseline concentrations of acylated ghrelin in the LP (200.26±100.35) vs. the FP (145.78±31.28), though this difference in baseline values between phases has not been reproduced in other work (Dafopoulos et al. 2009; McKie et al. 2018), and the two were not correlated post-exercise, making establishing a relationship between these variables challenging. Independent of phase, we observed a significant elevation of acylated ghrelin at 90 min post-exercise (243.27±175.24 $pg \cdot mL^{-1}$) compared to both immediately-post (136.96±86.80 $pg \cdot mL^{-1}$) and 30 min post-exercise (136.92±86.70 $pg \cdot mL^{-1}$), as is expected due to the rise in acylated ghrelin that occurs during periods of fasting (Murphy and Bloom 2006).

PYY_{3-36}

Contrary to our hypotheses, VICT did not affect PYY_{3-36} in either phase, as we observed no change immediately post-exercise (0.55±0.15 ng·mL^{-1}) vs. pre-exercise (0.51±0.13 ng·mL^{-1}). This finding is in contrast to the only other report examining the effect of VICT on appetite-regulatory hormones in females demonstrating a significant increase in PYY_{3-36} following a bout of running at 85% $\dot{V}O_{2max}$ (Howe et al. 2016), and to other work suggesting a greater post-exercise increase in women compared to men (Hagobian et al. 2013). However, as with acylated ghrelin, the research in females is limited, and other studies examining the impact of acute exercise of various intensities on PYY_{3-36} and total PYY in females have produced equivocal findings of increased (Hagobian et al. 2013; Hazell et al. 2017; Larson-Meyer et al. 2012) or unchanged (Alajmi et al. 2016; Hallworth et al. 2017; Panissa et al. 2016) concentrations post-exercise. Of note is the apparent sex difference in the total PYY response following MICT (30 min cycling at 65% $\dot{V}O_{2max}$) and SIT (six 30 s "all-out" efforts), where males demonstrated a greater post-exercise increase compared to females in the FP (Hagobian et al. 2013; Hazell et al. 2017; Larson-Meyer et al. 2012). The lack of response to exercise demonstrated in females is in line with the current study, as exercise of a vigorous intensity did not alter PYY_{3-36} post-exercise in either phase. Our novel observation in the LP suggests that neither exercise nor sex hormones appear to affect PYY_{3-36}, however further research is required to determine the reproducibility of our findings and reconcile the discrepancies with previous literature. Though preliminary, the lack of response to exercise in the LP is also in line with the hypothesis of preservation of reproductive function, as it would suggest that exercise is not successful in creating (or maintaining) an anorexigenic environment during a period of time important to child-bearing in women. Similarly, it has been suggested that the anorexigenic effects of E_2 are blunted in the presence of P_4, further

promoting an orexigenic environment in this phase (Frankovich and Lebrun 2000). However, this speculation must be interpreted with caution, as we also did not observe an increase in PYY_{3-36} in the FP, a time when energy intake tends to be lower in females, and no correlations were observed between sex hormones and PYY_{3-36}.

Active GLP-1

There were no differences in baseline concentrations of active GLP-1 between phases, as has been described in previous work in total GLP-1 (Brennan et al. 2009). Interestingly, active GLP-1 concentrations were significantly elevated at 30 min post-exercise (14.62±4.44 $pmol \cdot mL^{-1}$) compared to 90 min post-exercise (8.86±3.47 $pmol \cdot mL^{-1}$), which is in partial accordance with our hypothesis, albeit delayed as we hypothesized an increase would occur immediately post-exercise. The increase in active GLP-1 appears to be an exercise-induced effect due to the well-established fluctuations of GLP-1 between meals, such that plasma concentrations rise after a meal and are reduced with fasting (Murphy and Bloom 2006). However, other reports demonstrating an increase in total GLP-1 in females occurred immediately post-exercise (Hallworth et al. 2017; Howe et al. 2016; Larson-Meyer et al. 2012; Martins et al. 2007) and our observation of a delayed elevation has not been reported elsewhere. It is important to note that all prior observations of GLP-1 in females involved total GLP-1, whereas the present study measured active GLP-1. It is possible that this distinction underlies the discrepancy in results, as alternative forms of this peptide may respond differently to acute exercise (Hazell et al. 2016), though future work is warranted.

As with PYY, a distinct sex difference in the GLP-1 response to acute exercise has been observed in previous work, where total GLP-1 increased immediately following both MICT (30 min cycling at 65% $\dot{V}O_{2max}$) and SIT (six 30 s "all-out" efforts) compared to control levels in females only (Hagobian et al. 2013; Hazell et al. 2017; Larson-Meyer et al. 2012). Similar to PYY, Hazell and colleagues' observation of GLP-1 in the FP is in line with our current findings, demonstrating an increase in active GLP-1 regardless of phase, despite the difference in time-course.

Appetite Perceptions and Energy Intake

Contrary to our hypotheses, we observed no difference in overall appetite between the FP and LP, nor did we observe the well-described phenomenon of "exercise-induced anorexia" that typically occurs concomitantly with post-exercise decreases in acylated ghrelin and increases in satiety hormones (Deighton and Stensel 2014; Dorling et al. 2018). Similarly, no differences in free-living energy intake on the day of, or on the day following exercise were observed, and no differences between phases were found. Although many studies report simultaneous changes in these parameters, the divergence of the appetite hormone and perceptual response is not unique and has been reported at the group level in previous literature (Deighton et al. 2013; Martins et al. 2015; Sim et al. 2014). Furthermore, divergent responses at the individual level (despite a significant group change) has been highlighted in recent work examining individual variation in appetite suppression (Goltz et al. 2018), in addition to demonstrating true variability in the magnitude of the responses to exercise. As such, it is possible that some participants may have demonstrated the typical concomitant change in appetite hormones and perceptions while the majority of participants demonstrated a divergent response, resulting in the lack of overall change.

However, we are unable to assess individual variability in the current study due to the chosen experimental design of a single pair of trials (Hopkins 2015). Alternatively, it is possible that the observed change in acylated ghrelin (and lack thereof in satiety hormones) in the current study was not of sufficient magnitude to alter subjective appetite perceptions, and thus did not translate into a decrease in overall appetite. Lastly, other variables not examined in the current study may have influenced participants' perceived appetite. In this regard, it is important to note that the human appetite system is highly complex. Many other variables such as environmental cues or cognitive states are known to affect the control of energy intake (Murphy and Bloom 2006; Stensel 2010), and the hormonal response to exercise may be of lesser importance than other, non-homeostatic variables (Hagobian et al. 2013). Seemingly in line with this notion, while reduced absolute energy intake following an acute bout of exercise has been reported by others in males (Islam et al. 2017; Sim et al. 2014), it is uncommon, as the majority of current evidence suggests that single bouts of moderate- to high-intensity exercise do not alter free-living, absolute energy intake immediately post-exercise (Schubert et al. 2014). There is less agreement about the effect of chronic exercise on energy intake, as partial compensation (~30%) over a longer period of time (7 – 14 d) has been reported in both males (Stubbs et al. 2004; Whybrow et al. 2008) and females (Stubbs et al. 2002), suggesting more frequent bouts or longer exposures may be required to alter free-living energy intake. Further work is necessary to elucidate the long-term changes to energy intake following chronic exercise in both men and women.

Limitations

While this study provides valuable information regarding the effects of acute exercise on appetite regulation across the menstrual cycle, it is important to highlight several limitations. As

per the design of our study, we are unable to determine a causal relationship between sex hormone concentration and changes observed to appetite-regulatory hormones across the phases, and speculation of their involvement should be interpreted with caution. We did not examine energy intake in each phase during a control period (i.e., without exercise), and thus are unable to compare typical energy intake between phases due to the replication of 24 h intake between subsequent sessions. Though the timing of our sessions suggests data collection occurred during the appropriate phases (day 8 vs. day 22 on average), the baseline concentrations of P_4 in the FP suggest testing occurred in the late FP, thus we did not achieve a comparison between the minimum and peak values of this hormone, as was intended. Due to the relatively greater levels of P_4 observed during the FP, testing should occur earlier in this phase to ensure the lowest levels of both E_2 and P_4 are present, allowing for more accurate representations of the different responses between phases. Slightly higher than desired intra-assay coefficients of variation (>10%) were reported for P_4 and acylated ghrelin, however we were unable to perform new assays due to time constraints. This variability should be noted when interpreting the results, though is subject to change with the addition of the remaining 3 participants. Furthermore, as we did not measure hematocrit and hemoglobin, we were unable to correct exercise-induced changes in hormone concentrations for plasma volume shifts with exercise (Dill et al. 1974), though there is no consensus regarding the need for this procedure (Kargotich et al. 1998), and numerous appetite studies have reported a negligible influence of this correction on their findings (Douglas et al. 2017; Goltz et al. 2018; King et al. 2015). Lastly, though we have 3 remaining participants to include in the hormonal analysis, our sample size is relatively small, and their characteristics (healthy, recreationally active, naturally menstruating) limit the generalizability of our findings to

hormonal birth control users, post-menopausal women, overweight/obese, or sedentary populations.

Conclusion

To the best of our knowledge, this is the first study to examine appetite-regulatory and sex hormonal responses to VICT between the follicular and luteal phases of the menstrual cycle in healthy, recreationally active, naturally menstruating women. We observed a transient increase in E_2 in both phases post-exercise, as well as a trend of increased P_4 in the LP only. In addition, we showed lower a concentration of acylated ghrelin in the FP compared to the LP, with a trending suppression post-exercise that was phase-specific to the FP, potentially suggesting the acute appetite-regulatory response to exercise is blunted in the LP of the menstrual cycle. Lastly, both PYY_{3-36} and active GLP-1 responded similarly between phases, demonstrating no change or a delayed increase at 30 min-post exercise, respectively, suggesting sex hormones may not play a role in their responses to acute exercise. Given the small sample size of the current study, the novelty of these observations, and the lack of other studies conducted in the LP, future work should focus on the reproducibility of our findings to further elucidate the influence of sex hormones on appetite regulation across the menstrual cycle.

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CHAPTER 3:
GENERAL DISCUSSION & FUTURE DIRECTIONS

The current study examined the effects of acute exercise on acylated ghrelin, PYY_{3-36}, active GLP-1, subjective appetite perceptions, and free-living energy intake across the menstrual cycle to examine whether the presence of elevated E_2 and P_4 in the LP (exacerbated by vigorous-intensity exercise) influenced these effects. This work served as an important next step to previous work identifying clear sex differences in the appetite-regulatory response to acute exercise (Hagobian et al. 2009; Hazell et al. 2017; Larson-Meyer et al. 2012) and allowed us to more accurately characterize this response in the female population by examining the LP, as research is this field has been exclusively carried out in the FP. The results of this study contribute to a more complete understanding of the relationship between exercise and appetite control by examining females throughout the entire menstrual cycle, as per several important findings. We found that the well-documented post-exercise suppression of acylated ghrelin (Schubert et al. 2014) may be phase-dependent, as acylated ghrelin levels were significantly lower in the FP compared to the LP, with a greater post-exercise suppression in the FP, suggesting a blunted response in the LP. In addition, we found no observable effects of exercise on PYY_{3-36}, overall appetite, or free-living energy intake in either phase, as well as an increase in active GLP-1 at 30 min post-exercise (relative to 90 min post) independent of phase, suggesting the presence of elevated E_2 and P_4 in the LP does not influence these parameters. While these findings indicate menstrual cycle phase may play only a small role in appetite regulation across the menstrual cycle, our results are preliminary, and further work is necessary to explore these effects. In addition, there remain several important points that must be highlighted in light of our findings.

First, we aimed to create a unique paradigm to examine the potential effects of ovarian sex hormones on appetite-related parameters by implementing a vigorous-intensity exercise protocol that would selectively increase plasma concentrations of P_4 in the LP while increasing plasma E_2

in both the FP and LP, as per previous literature (Bonen et al. 1991; Jurkowski et al. 1978). In doing so, we hoped to create an environment that would allow for a unique comparison of the appetite-regulatory response in the presence of low P_4 and increased E_2 (the FP), and elevated levels of both E_2 and P_4 (the LP). While these patterns are what naturally occurs in each respective phase, it was our hope that the use of VICT would amplify these responses and allow for a clearer distinction of any potential between-phase differences. Though we did not observe an exercise-induced increase in P_4 during the LP as was expected, the natural cycling of P_4 between phases (and confirmed significant differences in baseline concentrations) allowed for a comparison between two distinct hormonal environments, as was the main objective of the overall project. In addition, if sex differences in the relationship between exercise and appetite hormones manifest only concurrently with peak concentrations of P_4, then they are likely inconsequential to overall energy balance due to the limited amount of time during which peak concentrations are present. It is possible that the elevation of ovarian sex hormones in previous work was a result of both an intensity and duration effect, as per the design of their exercise protocols, whereby exercise of light intensity was immediately followed by that of moderate and vigorous intensity until physical exhaustion (Bonen et al. 1991; Bonen et al. 1979; Jurkowski et al. 1978; Redman et al. 2003). As such, the lack of response in P_4 to VICT may have been a reflection of the shorter duration of exercise implemented in the current project. Additionally, the use of VICT precludes generalization of our findings to exercise of lesser intensities (i.e., moderate-intensity continuous training, or MICT), or to that of intermittent-style training, such as high-intensity interval training (HIIT) or sprint interval training (SIT). Further research is necessary to elucidate the appetite-regulatory response in the LP following various exercise protocols, and work regarding this topic is currently undergoing in our laboratory.

Second, as per the relatively small sample size in the current project and calculations of observed power, caution should be taken when interpreting the results as it is possible that a Type II error may have occurred in instances where no differences were detectable. For example, though there was no main effect of phase (P=0.973) or time (P=0.305) for changes in PYY_{3-36} relative to baseline, the observed power for each main effect equaled to 0.07 and 0.282, respectively, suggesting there is a large chance (~72-98%) that we failed to detect an effect that was present. Based on our observed effect size of f=0.18 in regard to the main effect of phase, a sample size of 86 participants would have been required to detect a difference in PYY_{3-36} between phases with 80% power. As the sample size of the current project (n = 8 for hormonal data) was well below this threshold, we are unable to conclude with a high level of certainty that the observed effect was representative of what would occur in the actual population. Similarly, to detect a difference in changes in PYY_{3-36} over time relative to baseline with 80% power and based on the observed effect size (f=0.41) for the main effect of time, 18 participants would be needed. Thus, while we have concluded that menstrual cycle phase and exercise appear to have no effect on PYY_{3-36} levels, this conclusion should be interpreted in the context of our statistical results. As per acylated ghrelin, the observed power for the main effect of phase and time equaled to 0.652 and 0.879, respectively, and that of the main effect of time for active GLP-1 equaled to 0.733, suggesting greater confidence can be placed that the effects observed in these hormones are what we would expect to occur in the actual population. In regard to the sex hormones, the observed power for the main effect of phase and time for E_2 was 0.44 and 0.71, respectively, and for that of the main effect of phase for P_4 equaled to 0.77. Nevertheless, while it is important to consider the findings of the current project in the context of our statistical results, the importance of the observed effects should not

be discounted, as they provide a starting point for understanding the effects of appetite regulation in the LP.

In addition, while it appears that sex hormones may play a role in the orexigenic response to exercise, the mechanisms involved in this effect remain to be elucidated. As aforementioned, E_2 may be directly involved in the regulation of ghrelin expression, as evidenced by an increase in ghrelin mRNA and plasma ghrelin concentrations following ovariectomy in rats that was reversed when normal levels of E_2 were restored (Matsubara et al. 2004). In addition, the role of E_2 in fat metabolism may influence ghrelin expression, as E_2 is known to promote lipolysis, thus increasing plasma free fatty acid concentrations and utilization of fat as substrate (Buffenstein et al. 1995). It is possible that this increase in fat utilization reduces the available O-octanoyl in circulation – the eight-carbon fatty acid required for acylation of ghrelin and thus, activation of its orexigenic effects. As such, due to its role in fat metabolism, an increased amount of E_2 in the FP may be indirectly associated with a decrease in plasma acylated ghrelin, and thus a concomitant reduction in perceptions of hunger post-exercise.

Only three previous studies have examined the effect of menstrual phase on appetite regulatory hormones (Brennan et al. 2009; Campolier et al. 2016; McKie et al. 2018), with two evaluating only anorexigenic hormones (Brennan et al. 2009; Campolier et al. 2016) and none evaluating the relationship between these hormones post-exercise. Based on this work, fasted/pre-exercise differences in acylated ghrelin or GLP-1 concentrations between phases were not expected. Campolier et al. (2016) observed lower fasted total PYY in the LP compared to the early FP, though the current investigation demonstrated no significant differences in PYY between phases at baseline. However, it is important to note that we examined PYY_{3-36} making it difficult to establish direct comparisons between other observations of total PYY. In the absence of

exercise or a test meal, the normal patterns of appetite hormone release are expected (see Chapter 1), as there is no evidence to suggest there are sex differences in the physiological mechanisms underlying short-term regulation of appetite (i.e. signaling mechanisms of gut peptides to the arcuate nucleus, the central integration of these signals, and the release of hypothalamic neuropeptides to modulate perceptions of hunger and satiety). Rather, existing evidence suggests potential differences between males and females is manifested in the appetite hormone responses to exercise, as outlined in Chapter 2, and that these differences may play a role in the difficulties females experience with weight loss from exercise.

Alternatively, such difficulties may not be related to the homeostatic response to exercise, and instead may be related to digestive responses across the menstrual cycle, as evidenced by the differential post-prandial responses across phases observed in the limited work examining appetite hormone response between phases. Brennan at al. (2009) observed delayed gastric emptying and lower total GLP-1 concentrations in the FP compared to the LP following ingestion of an oral glucose load, though a post-prandial difference between phases has not been reproduced in the other work available (McKie et al. 2018). Furthermore, in agreement with previous work (Brennan et al. 2009), Campolier and colleagues (2016) demonstrated slower gastric emptying in the FP compared to the LP, suggesting sex hormones may influence this digestive process, though total PYY was not directly associated with sex hormone concentrations. Similarly, we observed no correlations between PYY_{3-36} and sex hormones in the current study, again suggesting that sex hormones do not influence the anorexigenic appetite regulatory response to acute exercise in females. Further work is necessary to explore these preliminary findings.

Although a greater energy intake in the LP was expected due to the well-documented changes that occur in this variable across the menstrual cycle, the current investigation did not find

significant differences in total energy intake across 48 h between the FP and LP, excluding the day prior to each experimental session as this day was intentionally standardized between phases. This observation may be due to the fact that we utilized self-reported dietary logs as is a common strategy due to its feasibility, though is associated with greater variability between participants compared to standardized measures of energy intake (Jeacocke et al. 2010). In addition, energy intake is often underreported by ~25-50% using self-reported methods (Hise et al. 2002), and it is possible that the limitations associated with this method underlies our finding. Alternatively, a more apparent difference may have been found had we measured energy intake across each phase during a control period when no exercise was performed. Inclusion of this measurement may have provided a clearer picture of any effects of exercise on energy intake rather than to a standardized day across phases, and would have allowed for a direct examination of energy intake between phases in the absence of exercise. Future work should consider this distinction, and include a replicated crossover design with a control session to allow for a more thorough comparison of energy intake between phases.

Lastly, it should be noted that the hormones assessed in the current study do not provide a complete understanding of appetite regulation in humans, as the effects of peripheral appetite hormones are ultimately mediated by changes in hypothalamic neuropeptides discussed in Chapter 1. The majority of work examining these neuropeptides has been performed in animal models, and extension of this work in human models is a necessary step to understanding appetite regulation. Even further, this step is particularly important to understanding appetite-regulation across the menstrual cycle, as the rodent menstrual cycle is not an appropriate representation of that in women (Asarian et al. 2013).

Future Directions

As the first group to examine the appetite-regulatory response to acute exercise in the LP of the menstrual cycle, there are a number of avenues for further research that should be considered. First and foremost, as with any novel scientific finding, the reproducibility of our results should be examined, requiring replication of the current experimental design. Relatedly, in light of recent research demonstrating that true interindividual variability exists in the hormonal and subjective appetitive-response to acute exercise beyond that which would be expected due to measurement error and random variability (Goltz et al. 2018), future directions should focus on the assessment of interindividual differences in females using a replicated crossover design. A comparison of sex-based differences in interindividual variability may allow for a unique examination of whether females are more or less likely to compensate for an exercise-induced energy deficit compared to males, which may contribute to the weight loss difficulties females experience with exercise. Furthermore, importance should be placed on elucidating the independent roles of E_2 and P_4 on the neural control of feeding behaviours. Methodologically, it is not possible to isolate the effects of P_4 in naturally cycling women, as it is always present with increasing levels of E_2, however P_4 treatment in males may offer a unique avenue for distinguishing the independent effect of P_4 fluctuations on energy intake. Given the participant characteristics of the current project, examining exercise-induced changes in appetite hormones and appetite perceptions across the menstrual cycle in hormonal birth control users, post-menopausal women, overweight/obese and sedentary populations is needed. Lastly, future work should extend these findings to examine appetite responses in the LP following chronic exercise, as long-term energy balance ultimately dictates weight loss/gain. Overall, these questions will lead to an improved

understanding of the relationship between exercise and appetite control in women, providing more effective strategies for weight management.

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Appendix A

CONSENT TO PARTICIPATE IN RESEARCH Date:
LETTER OF INFORMATION

Title of Study: **Examining the potential involvement of sex hormones on appetite regulation (REB #5856)**

Dear _____:

You are being invited to participate in a research study conducted by Tom J. Hazell (PhD) and Sara Moniz (BA Kin) from the Energy Metabolism Research Laboratory. In order to participate it is important you are: a) between 18-30 years old, b) recreationally active <3/wk, c) healthy, d) normally menstruating, e) not currently pregnant, do not have plans to become pregnant during the duration of the study, and have not been pregnant (>3 months) within the last 3 years, and e) not using birth control or using no form of hormonal contraceptive as this project is designed to determine how sex hormones influence appetite hormones.

PURPOSE OF THE STUDY

The primary purpose of this study is to examine the effects of vigorous-intensity continuous running on concentrations of sex hormones and examine their associations with post-exercise alterations in appetite-regulating hormones in women during the follicular phase (FP) and luteal phase (LP) of the menstrual cycle.

PROCEDURES

This study requires you to visit the Energy Metabolism Research Laboratory 3 separate times, one for a familiarization session (~1 h 15 min) and then for 2 testing sessions (~4 h, one session in both the FP and LP) for a total time commitment of ~9 hours. The FP constitutes days ~1-11 of the menstrual cycle, including time of menses, and is the period of the cycle where levels of female sex hormones (estradiol and progesterone) are at the lowest concentration. The LP constitutes days ~16-28 of the menstrual cycle and is the period of time following ovulation (days ~12-15) where levels of estradiol and progesterone are increased relative to the FP. Menstrual phase will be subject-monitored through oral body temperature taken daily upon awakening, as well as through the use of at-home ovulation kits. Participants will be provided with an ovulation kit, instructed on its use, and will report the status of the test as well as body temperature each morning to the researcher via text or email. The familiarization session will include a test of cardiorespiratory fitness ($\dot{V}O_{2max}$ test), consisting of a graded running test performed until exhaustion. Following a 5 min warm up, you will begin the test running at a

speed between 5-7 mph at 0% incline. Every 2 minutes thereafter, incline on the treadmill will increase by 2%. The test will continue until volitional fatigue. All experimental sessions (separated by 1 week minimum) will include a standardized breakfast (consisting of a chocolate chip Clif bar) and 40 min exercise session (5 min warm-up, 30 min continuous running, and 5 min cool-down) followed by 2 hours post-exercise where participants rest comfortably and quietly in the laboratory (i.e. reading. A measurement of resting gas exchange will occur for 15 minutes before the exercise session, 30 minutes post-exercise, and for the final 15 minutes of the remaining 2 hours post-exercise. This measurement involves wearing a sanitized mask that is connected to a metabolic cart. Blood samples will also be drawn from the forearm pre- and post-exercise (two 3 mL samples per draw, 4 total blood draws). Blood draws will be taken from the inner elbow while lying supine by a trained researcher. All equipment will be sterile one-time use. Blood draw site will be cleaned prior to each sample and researcher will wear clean gloves to ensure cleanliness. You will also be asked several questions before and after exercise to determine your feelings of hunger and satiety as well as record dietary intake the day before the trial, on trial day and the day after. You will also be required to give a detailed history of your menstrual cycle, including the length of recent cycles, when you began menstruating, and if you have ever had any irregular menstrual cycles.

POTENTIAL RISKS AND DISCOMFORTS

We appreciate that this study requires you to discuss highly personal and sensitive information and we will do our best to make you as comfortable as possible; should you feel uncomfortable at any point you are free to withdraw from the study. All information regarding the menstrual cycle is only used to ensure that our testing days are done at the appropriate times.

Should this information be required for any presentations and/or publications, you will not identifiable by the information provided and results will be presented in group form. There is a possibility of mild muscle soreness and/or fatigue typical of an exercise session. You may feel some discomfort (lightheadedness, nausea, sore muscles) due to the intensity of the training or $\dot{V}O_{2max}$ test typical of strenuous physical exertion. Close monitoring of participants throughout the duration of the $\dot{V}O_{2max}$ test will limit this risk to ensure the test proceeds in a safe manner and will be immediately terminated should you display any signs of distress or upon your request. Risk of falling during the test will be limited as two researchers will always be present during testing. Although phlebotomy is safe when done by certified and trained 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 using a warm compress can alleviate this. 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 and/or fainting is minimum as the participant will be lying supine.

POTENTIAL BENEFITS TO SUBJECTS AND/OR SOCIETY

The potential benefits of your participation include a better understanding of your cardiorespiratory fitness based on the results of a $\dot{V}O_{2max}$ test, an assessment which typically costs ~$120 when performed at commercial facilities. Results from this study will further our

understanding of appetite regulation in females and may offer new insights into potential mechanisms involved in appetite regulation. Thus, participants will gain insight into how natural hormonal changes may affect their weight loss efforts and their eating habits which may lead to improved weight loss strategies for women.

CONFIDENTIALITY

All information obtained in connection with this study will be de-identified. **It is possible that data related to your participation (i.e., your $\dot{V}O_{2max}$ max value, hormone levels, appetite scores, energy intake data) and basic demographic data such as physical activity level, height, weight, and age) will be submitted to an 'open access' database upon publication. This data will be completely anonymized and de-identified by removing names and any other information that could possibly identify any participant.** 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. All results will be collapsed before analysis. All blood samples will be stored in secured location until analysis and subsequently destroyed after a period of 5 years. All other data will also be retained and destroyed after 5 years.

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.). Please note that should you withdraw from the study all personal information and blood samples will be immediately destroyed.

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 and include the preferred address for communication (i.e. email or mailing address). 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, 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. Jayne Kalmar, Research Ethics Board (REB) Chair (REBchair@wlu.ca / 519-884-0710 x 3131). This project has been reviewed and approved by the REB – Approval #5856.

Sincerely,
Sara C. Moniz (moni8339@mylaurier.ca), MKin Student, and Dr. Tom Hazell (thazell@wlu.ca), Associate Professor
Department of Kinesiology and Physical Education, Wilfrid Laurier University, Waterloo, ON

Title of Study: **Examining the potential involvement of sex hormones on appetite regulation (REB #5856)**

Consent Statement

Principal Investigators: Dr. Tom Hazell, Sara C. Moniz

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 B

EMRL
Energy Metabolism Research Laboratory

FEMALES NEEDED FOR APPETITE REGULATION STUDY!

What is appetite regulation?

- Certain hormones in our body control how much we eat
- The female menstrual cycle may alter these hormones

Purpose: To determine whether the hormones associated with the menstrual cycle (MC) alter the hormones that control food intake in different phases of the MC

Who can participate?

- Non-smoking, regularly menstruating females aged 18-30
- Individuals not using birth control or those using non-hormonal forms
- Not pregnant/no pregnancy within the past 3 years (> 3 months)
- Recreationally active (< 3 training sessions per week)

Time commitment:

- One familiarization session → ~ 1 h 15 min
- Two experimental sessions → ~ 4 h each

Details:

- You will be provided breakfast at the beginning of the session
- Involves 30 min of continuous running on a treadmill
- During the session blood samples will be drawn from your arms

If interested, please contact: **Sara Moniz (moni8339@mylaurier.ca)**

This project has been reviewed and approved by the REB (#5856)

Appendix C

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
		1 Have you experienced **ANY** of the following (A to F) **within the past six months**?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	**2** Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	**3** Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	**4** Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ☐ DAYS/ WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ☐ MINUTES/ DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ☐ MINUTES/ WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.

☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print]	Signature (or Signature of Parent/Guardian if applicable)	Date of Birth

Date	Email (optional)	Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

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Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered YES to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

1 Have you experienced ANY of the following (A to F) within the past six months?	
A A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity? ☐ YES	Physical activity is likely to be beneficial. If you have been treated for heart disease but have not completed a cardiac rehabilitation program within the past 6 months, consult a doctor – a supervised cardiac rehabilitation program is strongly recommended. If you are resuming physical activity after more than 6 months of inactivity, begin slowly with light- to moderate-intensity physical activity. If you have pain/discomfort/pressure in your chest and it is new for you, talk to a doctor. Describe the symptom and what activities bring it on.
B A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher? ☐ YES	Physical activity is likely to be beneficial if you have been diagnosed and treated for high blood pressure (BP). If you are unsure of your resting BP, consult a health care provider or a Qualified Exercise Professional (QEP) to have it measured. If you are taking BP medication and your BP is under good control, regular physical activity is recommended as it may help to lower your BP. Your doctor should be aware of your physical activity level so your medication needs can be monitored. If your BP is 160/90 or higher, you should receive medical clearance and consult a QEP about safe and appropriate physical activity.
C Dizziness or lightheadedness during physical activity ☐ YES	There are several possible reasons for feeling this way and many are not worrisome. Before becoming more active, consult a health care provider to identify reasons and minimize risk. Until then, refrain from increasing the intensity of your physical activity.
D Shortness of breath at rest ☐ YES	If you have asthma and this is relieved with medication, light to moderate physical activity is safe. If your shortness of breath is not relieved with medication, consult a doctor.
E Loss of consciousness/fainting for any reason ☐ YES	Before becoming more active, consult a doctor to identify reasons and minimize risk. Once you are medically cleared, consult a Qualified Exercise Professional (QEP) about types of physical activity suitable for your condition.
F Concussion ☐ YES	A concussion is an injury to the brain that requires time to recover. Increasing physical activity while still experiencing symptoms may worsen your symptoms, lengthen your recovery, and increase your risk for another concussion. A health care provider will let you know when you can start becoming more physically active, and a Qualified Exercise Professional (QEP) can help get you started.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire – Reference Document

ADVICE ON WHAT TO DO IF YOU HAVE A **YES** RESPONSE

Use this reference document if you answered YES to any question and you have not consulted a health care provider or Qualified Exercise Professional (QEP) about becoming more physically active.

2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active? ☐ YES

If this swelling or pain is new, consult a health care provider. Otherwise, keep joints healthy and reduce pain by moving your joints slowly and gently through the entire pain-free range of motion. If you have hip, knee or ankle pain, choose low-impact activities such as swimming or cycling. As the pain subsides, gradually resume your normal physical activities starting at a level lower than before the flare-up. Consult a Qualified Exercise Professional (QEP) in follow-up to help you become more active and prevent or minimize future pain.

3 Has a health care provider told you that you should avoid or modify certain types of physical activity? ☐ YES

Listen to the advice of your health care provider. A Qualified Exercise Professional (QEP) will ask you about any considerations and provide specific advice for physical activity that is safe and that takes your lifestyle and health care provider's advice into account.

4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active? ☐ YES

Some people may worry if they have a medical or physical condition that physical activity might be unsafe. In fact, regular physical activity can help to manage and improve many conditions. Physical activity can also reduce the risk of complications. A Qualified Exercise Professional (QEP) can help with specific advice for physical activity that is safe and that takes your medical history and lifestyle into account.

After reading the ADVICE for your YES response, go to Page 2 of the *Get Active Questionnaire* – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

WANT ADDITIONAL INFORMATION ON BECOMING MORE PHYSICALLY ACTIVE?

- **csep.ca/certifications**
  CSEP Certified members can help you with your physical activity goals.
- **csep.ca/guidelines**
  Canadian Physical Activity Guidelines for all ages.

Appendix D

Participant Code: _____

Date: _____

Session: _____

How often do you eat breakfast in the morning?

Never _____ **Every morning**

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**

How anxious do you feel?

Not anxious at all _____ **Very anxious**

How nauseous do you feel?

Not nauseous at all _____ **Very nauseous**

Appendix E

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.

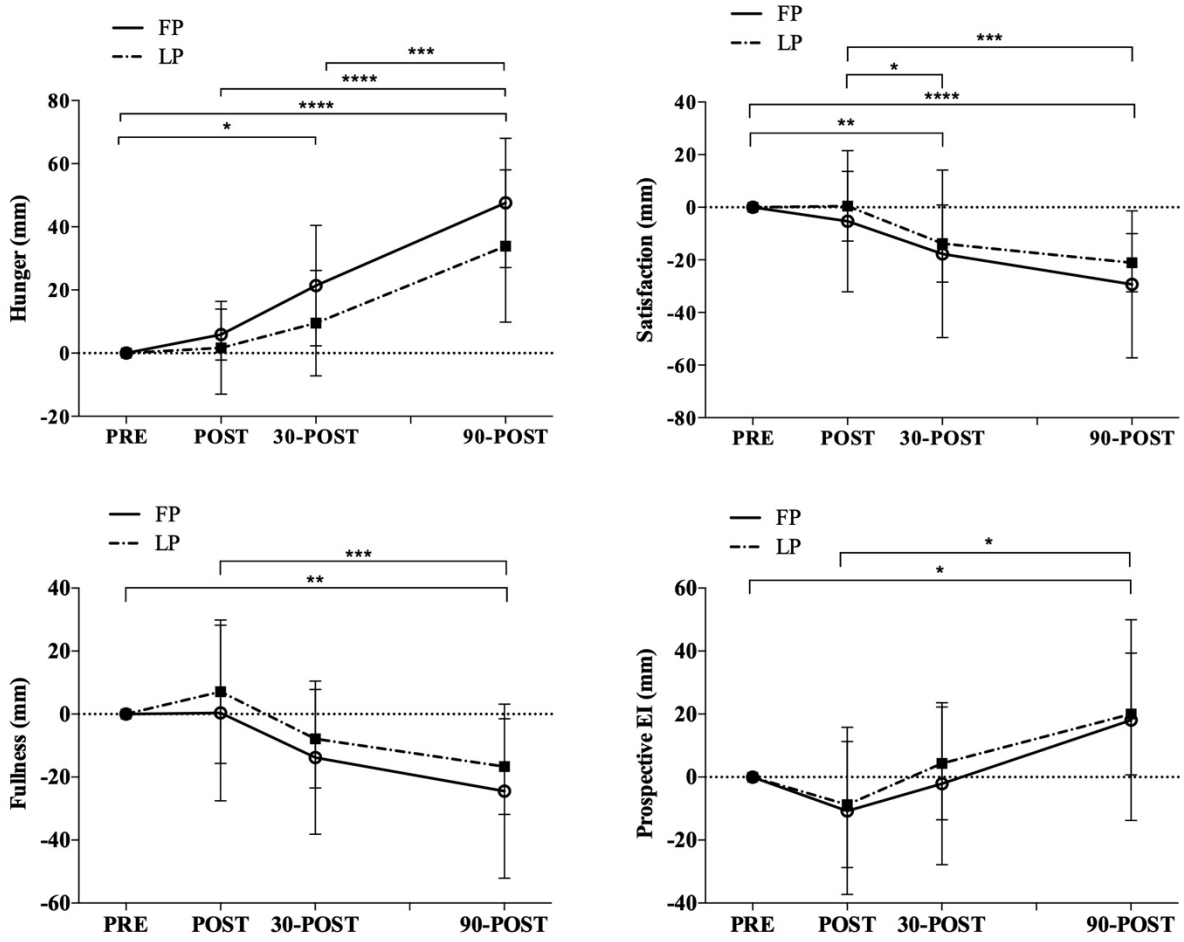
Example:

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	Domino's 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 F


FP
LP
Hunger (mm)
80
60
40
20
0
-20
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****
****
***
PRE
POST
30-POST
90-POST
FP
LP
Satisfaction (mm)
40
20
0
-20
-40
-60
-80
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*
****
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PRE
POST
30-POST
90-POST
FP
LP
Fullness (mm)
40
20
0
-20
-40
-60
**
***
PRE
POST
30-POST
90-POST
FP
LP
Prospective EI (mm)
60
40
20
0
-20
-40
*
*
PRE
POST
30-POST
90-POST

Appendix G

Table 1. Individual participant characteristics

Participant	Height (cm)	Weight (kg)	BMI	$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	GLTE-Q Score	Activity Levels
1	167.0	88.9	31.9	37.08	58	Active
2	165.0	57.6	21.2	38.10	27	Active
3	166.0	71.5	25.9	29.10	31	Active
4	163.4	52.5	19.7	41.40	18	Mod A
5	155.0	55.0	22.9	44.20	28	Active
6	163.2	59.1	22.2	35.30	37	Active
7	162.1	54.0	20.5	37.90	45	Active
8	162.0	64.2	24.5	39.80	38	Active
9	157.2	52.6	21.3	37.10	47	Active
10	166.0	64.2	23.3	37.10	35	Active
11	170.4	61.8	21.3	30.10	42	Active
MEAN ±SD	163.39 ±4.37	61.95 ±10.69	23.34 ±3.75	37.02 ±4.40	37 ±12	-

Note: Activity levels are based on the Godin-Leisure Time Exercise Questionnaire scoring index and interpretation, where a score: <14 = insufficiently active/sedentary; 14-23 = moderately active; >24 = active.