

**THE INFLUENCE OF EXERCISE INTENSITY ON APPETITE REGULATING  
HORMONES GLP-1 AND PYY IN ACTIVE HEALTHY ADULTS**

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## **Dedication**

This Thesis is dedicated to my parents, Rae and Jim, who through their example have gifted me the greatest education I will ever receive. Their continued support, emotionally and financially, and encouragement to never let hesitation get in the way of a dream have allowed me the opportunities I have today. They have always put my needs before their own, been my number one fans, and will always be my heroes. Also, a special dedication for Landon who has been a constant source of support and security during the challenges of graduate school. His never failing humor and wit made my days a little brighter and kept me going when didn't know I could. The comfort and mirth he brings me could never be matched and I will never be able to adequately express my appreciation for his care and encouragement along this journey. To those I love the most, thank you.

## **Abstract**

Exercise is a known contributor to fat loss, its efficacy increasing when combined with an energy-restricted diet in order to create an energy deficit. Claims exist that exercise intensity may influence one's hunger and energy intake post-exercise, affecting the efficacy of exercise for fat loss. Additionally, women may increase energy intake following exercise to compensate for the increased energy expenditure more than men. This study used a randomized crossover design to examine the effect of exercise intensity on appetite regulating hormones GLP-1 and PYY, and subjective hunger in women, and compared these results to men following endurance cycling. Both sprint interval cycling and continuous endurance cycling influenced the concentration of GLP-1 and PYY, though not differently, and had no influence on perceived hunger. There was no significant difference in the response of these satiety hormones and hunger between men and women following endurance cycling.

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## List of Abbreviations

AE: Aerobic exercise  
AgRp: Agouti-related peptide  
ANOVA: Analysis of Variance  
ARC: Arcuate nucleus  
BBB: Blood brain barrier  
BMI: Body mass index  
BMR: Basal metabolic rate  
CART: Cocaine and amphetamine-related transcript  
CNS: Central nervous system  
CSEP: Canadian Society of Exercise Physiology  
CTRL: Control  
EB: Energy balance  
EDTA: Ethylenediaminetetraacetic acid  
EE: Energy expenditure  
EF: Essential fat  
EI: Energy intake  
ELISA: Enzyme-linked immunosorbent assay  
END: Endurance  
ER: Energy regulation  
FFM: Fat-free mass  
FM: Fat mass  
GI: Gastrointestinal  
GLP-1: Glucagon-like peptide-1  
HIIT: High-intensity interval training  
HR: Heart rate  
MC3R: Melanocortin-3 receptor  
MC4R: Melanocortin-4 receptor  
NPY: Neuropeptide Y  
PA: Physical activity  
PAR-Q: Physical activity readiness questionnaire  
PNS: Parasympathetic nervous system  
POMC: Pro-opiomelanocortin  
PP: Pancreatic polypeptide  
PVN: Paraventricular nucleus  
PYY: Peptide tyrosine-tyrosine  
RER: Respiratory exchange ratio  
RMR: Resting metabolic rate  
RPE: Rating of Perceived Exertion  
SIT: Sprint interval training  
SNS: Sympathetic nervous system  
SPSS: Statistical Package for the Social Sciences  
TDEE: Total daily energy expenditure  
TRH: Thyrotropin-releasing hormone  
VAS: Visual analogue scale  
VO<sub>2max</sub>: Maximal oxygen uptake

## **Chapter 1: Literature Review**

### **Introduction**

Obesity rates continue to rise in Canada where nearly 34% of women and 46% of men report being overweight or obese, an increase of more than 15% between 2012 and 2014 (Statistics Canada, 2015). In order to improve the health and quality of life of Canadian citizens, obesity prevention strategies must be developed to promote healthy weight maintenance. Fat mass is maintained when the body is in a state of energy balance (EB); that is, when energy intake (EI) and energy expenditure (EE) are equal. Here, it is the composition of weight loss, with interest in fat loss, not total body mass that is of interest. Fat mass (FM) increases when the body is in a sustained state of positive EB; when EI is chronically greater than EE (Hall et al., 2012). Excess dietary calories from energy-dense convenience foods and sugar-laden beverages, coupled with a lack of physical activity (PA) contribute to sustained positive EB and Canada's obesity epidemic (Crespo, Cachero, Jimenes, Barrios & Ferreiro, 2014). Lifestyle strategies are required that promote increased EE and/or decreased EI to reduce the risk of obesity.

It is well known that exercise can create an acute negative EB, but there is a general lack of understanding as to how this relates to weight loss. Many individuals find weight loss and body fat loss from exercise alone to be extremely difficult (King et al., 2007), which is backed by research that indicates that solely increasing EE has minimal effects on fat loss (Church, Martin, Thompson, Earnest, Mikus & Blair, 2009; Donnelly et al., 2003; Miller, Koceja & Hamilton, 1997). Inaccuracies in one's estimation of their EI respective to exercise EE is a possible reason why individuals struggle with weight loss from exercise interventions alone (Westerterp & Goris, 2002). For example, increasing EI

to compensate for exercise EE often occurs, especially in individuals new to exercise, either as a reward for their efforts or out of a general misunderstanding of EI requirements following exercise (Dole, Wansink, & Zehnder, 2014; Finlayson, Bryant, Blundell & King, 2009). Another possibility is that acute bouts of PA elicit compensatory increases in EI via intrinsic protective mechanisms, guarding individuals against a sustained negative EB (King et al., 2007).

The regulation of EI is under both psychological and physiological control. Psychological and environmental factors are strongly associated with an individual's lifestyle habits and the drive to seek highly palatable foods for pleasure and enjoyment (King et al., 2007; Woods et al., 2004). These psychological influences often drive and override physiological mechanisms of human appetite and can only be measured by subjective ratings, making them difficult to quantify (Blundell & King, 1996; Mattes, 1990; Valassi, Scacchi, & Cavagnini, 2008).

Physiological regulation of appetite occurs in the arcuate nucleus (ARC) of the hypothalamus where peripheral anorexigenic (satiety) and orexigenic (appetite) hormones control neuropeptide release, which then elicit a cascade of signals causing one to experience either hunger or satiety (Lenard & Berthoud, 2008). These, coupled with psychological influences, ultimately determine eating behavior. The orexigenic hormone ghrelin triggers the release of neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the hypothalamus, and impairs the release of satiety related neuropeptides, eventually stimulating appetite. The anorexigenic hormones are: peptide tyrosine-tyrosine (PYY), pancreatic polypeptide (PP), leptin, and glucagon-like peptide-1 (GLP-1). These hormones signal the release of anorexigenic neuropeptides, including cocaine and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC), and inhibit

the release of NPY/AgRP in the ARC, to inhibit appetite and promote satiety (Kampe, Tschop, Horvath & Widmer, 2010).

In women, cyclical fluctuations in EE and EI have been shown across the various phases of the menstrual cycle (Abraham, 1983; Ferraro et. al., 1992; Howe, Rumpler & Seale, 1993; Webb, 1986). Although small, a decrease in EE during the follicular phase has been observed, likely caused by changes in resting metabolic rate (RMR) as a result of fluctuations of in ovarian steroid hormones (Bisdee, James & Shaw, 1989; Buffenstein, Poppitt, McDevitt, & Prentice, 1995). Additionally, EI has been found to be lower during the follicular phase compared to postovulatory and premenstrual phases (Dye & Blundell, 1997). In contrast, EI has been found to increase, trending towards significance, during the early follicular phase in some women (Wurtman, Brzezinski, Wurtman & Laferrere, 1989). Differences in participant's age, body weight and use of oral contraceptives may be factors that contribute to the dissimilarities in these results. These variations in EE and EI across the menstrual cycle are not observed in energy-restricted populations or women using oral contraceptives (Anantharaman-Bar , Clavien, Grmender & Pollett, 1988; Schweiger et al., 1992). It is likely that changes observed in EI across the cycle are related to changes in basal metabolic rate (BMR) that occur over the course of the menstrual cycle (Solomon, Kurzer & Calloway, 1982; Webb, 1986). Alterations in BMR likely occur with various hormonal shifts, which influence both EI and EE independently. At this time, it is unclear if these variations translate to changes in total EB.

Orexigenic and anorexigenic hormones and their effects on the hypothalamic ARC region are known to be influenced by both meal consumption (caloric load and macronutrient composition) and fasting (Perry & Wang, 2012). What is less understood is how exercise affects this pathway and whether the response to exercise differs between

sexes. Women may present a different response to exercise compared to men in attempt to protect against negative EB, preserving essential body fat for reproductive purposes (Hagobian et al., 2008). Women typically carry ~10% greater body fat compared to men across the lifespan, as an evolutionary protective mechanism for reproductive health (Jackson et al., 2002; Womersley & Durin, 1977). Reproduction in females requires a significantly greater EE than males and in times of negative EB it is essential that women have oxidizable substrate available for both reproductive purposes and essential life functions (Bronson, 1989; ESHRE Capri Workshop Group, 2006). Currently, very little of the research on exercise-induced effects on appetite hormones has included female populations. The purpose of this literature review is to explore our current knowledge of the effects of exercise on the neuroendocrine control of EI, including the response in healthy women.

## **Energy Intake Regulation**

### **Peripheral Gut Hormones: Orexigenic**

**Ghrelin.** The only known circulating appetite hormone, ghrelin, is a potent stimulus for feeding. Ghrelin is produced primarily by specialized cells in the gut and is also released in the duodenum, ileum, cecum and colon (Cummings et al., 2002; Date et al., 2000; Kojima et al., 1999). Resting ghrelin levels are inversely associated with adiposity and are elevated in a fasted state, decreasing post-prandially in proportion to the caloric load and circulating micronutrient signals (i.e. glucose) of a meal (Ariyasu et al., 2001; Callahan et al., 2004; Cummings et al., 2002; Stanley, Wynne, McGowan & Bloom, 2005). Ghrelin levels are also influenced by satiety hormone leptin and fluctuate

with the diurnal variation of leptin concentration, where the lowest concentrations of leptin and highest levels of ghrelin occur in the morning (Cummings et al., 2002).

Ghrelin is found in peripheral circulation in two forms: acylated and desacyl ghrelin (Hosoda, Kojima, Matsuo & Kangawa, 2000). Acylated ghrelin has the addition of an octanoyl group, allowing it to cross the blood-brain barrier (BBB) and act on the central nervous system (CNS), so the octanoylation is essential for ghrelin's effect on EI (Kojima et al., 1999). Both acylated and des-acyl ghrelin act upon growth hormone secretory receptors of NPY/AgRP arcuate neurons, stimulating the up-regulation of NPY and AgRP, eventually increasing EI (Mason, Wang & Zigman, 2013). Prior to the development of an assay for plasma acylated ghrelin in humans, only studies of total ghrelin were possible. The measurement of total ghrelin does not provide an accurate representation of changes in the active form and therefore, when studying the effect of exercise on appetite stimulation, investigations of the active acylated form of ghrelin are more informative for neuroendocrine EI regulation (Mackelvie et al., 2007). While acylated ghrelin may be more relevant for neuroendocrine energy regulation (ER), it has been suggested that total ghrelin may also influence appetite through a cascade of signals that increases neural activity in the hedonic brain areas, enhancing the desire to seek appetizing foods for pleasure rather than true hunger (Perello et al., 2010; Wren et al., 2000). As such, there is a place in ER research for measurement of both acylated and total ghrelin activity. In addition to the initiation of eating behaviors, an elevated pre-prandial level of ghrelin has been positively correlated with increased subjective hunger scores in anticipation of food in human research (Cummings, Frayo, Marmonier, Aubert & Chapelot, 2004). There may be a conditioned response to feeding, preparing the metabolism for the influx of calories (Stanley et al., 2005).

While little research in ER has been performed on female participants, it is important to note that there is a possible sex difference in plasma ghrelin levels, though research is conflicting. Ghrelin levels have been shown to be comparable in healthy, normal-weight males and females (Bellone et al., 2002; Purnell, Weigle, Breen & Cummings, 2003; Stylianou et al., 2007; Tschop et al., 2001). However, additional research has shown higher fasting levels in females compared to males (Barkan, Dimaraki, Jessup, Ermolenko & Jaffe, 2003; Makovey, Naganathan, Seibel & Sambrook, 2007; Schuessler et al., 2005). Higher plasma ghrelin levels have been found in women and are inversely correlated with body mass index (BMI), FM, and related to fat-free mass (FFM) in females but not males (Abu-Farha et al., 2014; Greenman et al., 2004; Makovey et al., 2007). It is possible that the higher concentration of ghrelin measured in females acts as a protective mechanism for female reproductive health, guarding against negative EB and ensuring sufficient energy for child bearing (Hagobian et al., 2008).

### **Peripheral Gut Hormones: Anorexigenic**

**Peptide tyrosine-tyrosine (PYY).** This peptide hormone is produced by the distal-intestinal L cells of the gastrointestinal (GI) tract (Cummings & Overduin, 2007; Eberlein et al., 1989), with expression increasing in the ileum, colon and rectum (Adrian et al., 1985; Ekblad & Sunder, 2002). PYY is found in low concentration in a fasted state and high concentration following a meal in proportion to caloric load and macronutrient composition of the meal (Batterham et al., 2002; Lin & Chey, 2003). Increases in PYY are seen immediately following ingestion of a meal (suggesting the involvement of a neural reflex), plateauing within 1- to 2-h, but staying elevated 6-h following a meal (Adrian et al., 1985). PYY exists in two forms, PYY<sub>1-36</sub> and PYY<sub>3-36</sub>. The active form

(PYY<sub>3-36</sub>) travels to the hypothalamic ARC after crossing the BBB and binds to NPY-Y2 receptors on NPY/AgRP neurons (Cummings & Overduin, 2007). This binding inhibits NPY mRNA expression and elicits numerous other actions, including: preventing downstream inhibition of alpha melanocyte-stimulating hormone (alpha-MSH) release by AgRP, blocking the binding of orexigenic hormones, increasing expression of ARC POMC neurons, and decreasing EI (Batterham et al., 2002; Batterham et al., 2003; Challis et al., 2003; DeSilva & Bloom, 2012; Lumb et al., 2007; Nonaka, Shioda, Neihoff & Banks, 2003; Stanley et al., 2005). PYY also satiates by causing a delay in gastric emptying, reducing gastric and pancreatic secretion, and increasing ileal absorption of electrolytes and fluids (Allen et al., 1984; Adrian et al., 1985; Ashby & Bloom, 2007; Hoentjen, Hopman & Jansen, 2001).

Sex differences may exist in both fasting and postprandial concentrations of PYY, though results are conflicting. Compared to males, females have higher fasting PYY concentrations, although time to peak PYY concentration after feeding is similar in both sexes (Kim et al., 2005). In contrast, both fasting and postprandial concentrations of PYY have been measured to be lower in females (Essah, Levy, Sistrun, Kelly & Nestler, 2006). Differences in the circulating concentrations of PYY in the current literature may exist due to differences between studies in the body fat levels of the participants, as body composition and EB have been shown to influence resting PYY concentration (Cahill et al., 2014).

**Pancreatic polypeptide (PP).** Another anorexigenic peptide hormone, PP is released from gamma cells of the pancreas, located in the Islet of Langerhans (Larsson, Sundler & Hakanson, 1975). PP concentrations are lowest in the early morning and highest in the evening, with feeding also influencing PP secretion for up to 6-h (Track,

McLeod & Mee, 1980). Total release of PP increases in proportion to caloric intake following a meal, however, the increase in PP has also been shown to be biphasic; as circulating levels continue to increase with consecutive meals (Adrian, Bloom, Bryant, Polak & Heitz, 1976; Asakawa et al., 1999; Track et al., 1980). Circulating levels of PP are also increased by motilin and gastric distension and are found to be representative of energy stores, with circulating concentration inversely proportional to fat mass (Arosio et al., 2003; Christofides et al., 1979; Fujimoto et al., 1997; Mochiki, Inui, Satoh, Mizumoto & Itoh, 1997; Peracchi, Tagliabue, Quatrini & Reschini, 1999; Uhe et al., 1992). Anorexigenic effects of PP may be mediated by NPY-Y4 and Y5 receptors in the hypothalamus and brainstem (Asakawa et al., 2003). Peripheral administration of PP in ob/ob obese mice causes decreased NPY and ghrelin concentration, decreased food intake and gastric emptying, and provokes a negative EB sufficient enough for reduction in body weight (Asakawa et al., 2003). Conversely, direct administration of PP to the third ventricle increased EI in rats (Clark, Kalra, Crowley & Kalra, 1984). It is not known if the same response occurs in humans. Currently, the mechanism by which PP controls food intake is not well understood, and it is not known if PP secretion or action differs between males and females.

**Glucagon-like peptide (GLP-1).** GLP-1 is secreted from the L cells of the small intestine, co-localized with PYY (Cummings & Overduin, 2007; Kreymann, Ghatei, Williams & Bloom, 1987). The two have additive effects on EI inhibition (Neary, Goldstone & Bloom, 2004). GLP-1 exists primarily in two forms in humans, the majority of the circulating biologically active form being GLP-1<sub>7-36</sub> with smaller amounts of glycine extended GLP-1<sub>7-37</sub> (Kim & Egan, 2009). Following its secretion, GLP-1<sub>7-36</sub> is rapidly metabolized to GLP-1<sub>9-36</sub>, which is the predominant form of GLP-1 postprandially

(Vahl, Paty, Fuller, Prigeon & D'Alessio, 2003). Although there is limited evidence for the biological role of GLP-1<sub>9-36</sub> it has been suggested that it promotes glucose metabolism in peripheral tissue and antagonizes the effects of intact GLP-1 (Vahl et al., 2003). Circulating concentrations of GLP-1 are inversely associated with body fat mass and are released into circulation following a meal in proportion to the amount of energy consumed (Holst, Schwartz, Lovgreen, Pedersen & Beck-Nelson, 1983; Naslund et al., 1999; Ranganath et al., 1996; Verdich et al., 2001). It acts to inhibit further EI, though the effect is small (Kreymann et al., 1987; Verdich et al., 2001). Inhibition of EI occurs by causing a delay in gastric emptying when GLP-1 binds to receptors disbursed throughout the brain, pancreas and GI tract (Cummings & Overduin, 2007; Yu & Kim, 2012). In addition, GLP-1 stimulates insulin secretion and helps maintain glucose homeostasis (Small & Bloom, 2004; Tang-Christensen, Vrang, & Larsen, 2001). Sex differences may exist in GLP-1 secretion; however, to the author's knowledge there is no research investigating this.

**Leptin.** Leptin is a hormone produced by adipocytes and the lower gastric epithelium and its' concentration is related to both acute EB, energy stores and fat mass (Bado et al., 1998; Considine & Caro, 1997; Maffei et al., 1995; Zhang et al., 1994). It is a peptide hormone that crosses the BBB and communicates with both populations of appetite regulating neurons in the ARC, amongst other brain areas (Pinto et al., 2004). Leptin suppresses appetite and increases EE by facilitating POMC/CART-expressing neurons and inhibiting orexigenic NPY/AgRP neurons (Elmqvist, Maratos-Flier, Saper & Flier, 1998; Myers, Cowley & Munzberg, 2008; Schwartz, Woods, Porte, Seeley & Baskin, 2000). When food is restricted, leptin concentrations are low and NPY/AgRP are

upregulated as a result. In contrast, leptin concentrations are high in a fed state and POMC/CART expression is upregulated (Huang, Ham, South & Storlien, 2003).

Plasma concentrations of leptin show clear sex differences, likely due to differences in body fat percentage, with females having significantly greater levels of plasma leptin compared to males (Ahmed et al., 1999; Azar, Salti, Zantout, Shahine, & Zalloua, 2002; Chan et al., 2002; Nakanishi et al., 2001; Stylianou et al., 2007).

### **Neuropeptides: Orexigenic**

**Neuropeptide Y (NPY).** Neuropeptide Y is the strongest orexigenic neuropeptide known and is synthesized in NPY/AgRP neurons of the hypothalamic ARC (Allen et al., 1983; Hahn, Breininger, Baskin & Schwartz, 1998). NPY expression increases with fasting and decreases after feeding (Kalra, Dube, Sahu, Phelps & Kalra, 1991; Sanacora, Kershaw, Finkelstein & White, 1990; Swart, Jahng, Overton & Houpt, 2002). The orexigenic signal travels from the ARC to the paraventricular nucleus (PVN) and then on to further brain centers, specifically binding to Y1 and Y5 receptors (Cabrele et al., 2000; Kask, Rago & Harro, 1998). Both Y1 and Y5 receptor density decrease with fasting (Cheng et al., 1998; Widdowson, et al., 1997). Elevated expression of NPY in the PVN results in a surplus of efferent responses which increase EI and decrease EE, including: increased appetite, decreased PA, suppression of the sympathetic nervous system (SNS), activation of the parasympathetic nervous system (PNS), inhibition of the thyroid axis, and inhibition of brown fat thermogenesis (Billington, Briggs, Grace & Lecine, 1991; Fekete et al., 2002; Frankish, Dryden, Hopkins, Wong & Williams, 1995). Together, these cause positive EB.

There have been no studies to date that investigate sex differences in NPY expression and ER. As such, little is known about whether NPY differs between males and females relative to EB status, however, NPY expression has been shown to be influenced by the sex steroids. Hypothalamic expression of NPY is affected differentially by androgens and estrogen (Clegg et al., 2006; Pelletier, Li, Luu-The, & Labrie, 2007; Sohn, Wolden-Hanson & Matsumoto, 2002). Estrogen plays a role in appetite regulation by decreasing the action of NPY (Messina, Boersma, Overton & Eckel, 2006; Wade & Gray, 1985). There are no differences in basal NPY concentrations between sexes, though stress can cause a sexually differentiated NPY expression (Zukowska-Grojec, Shen, Caparo, & Vaz, 1991). Considering the role of estrogen and stress hormones on NPY activity, there is the potential for women to have suppressed action of NPY in a state of positive EB compared to men. This has yet to be researched.

**Agouti-related protein (AgRP).** An orexigenic peptide that is released from neuronal cells in the ARC, AgRP strongly influences EI (Broberger, Johansen, Johansson, Schalling & Hokfelt; 1998; Hahn et al, 1998; Schwartz et al., 2000). AgRP acts as an endogenous antagonist for both melanocortin-3 and -4 receptors (MC3R and MC4R), which are G-protein coupled receptors that are part of the anorexigenic melanocortin system (Lavebratt, 2007; Ollmann et al., 1997). Expression of AgRP is increased by fasting, and unlike NPY, levels remain elevated for prolonged (>6-h) periods (Swart et al., 2002). Due to this prolonged response, AgRP has a stronger cumulative influence on EI than NPY (Hagan et al., 2000). Antagonistic binding of AgRP to MC4R receptors directly elicits an orexigenic effect (Parker & Bloom, 2012). AgRP has also been shown to indirectly stimulate an orexigenic effect when centrally administered, blocking the agonistic binding of anorexigenic alpha-MSH at MC3R receptors (Parker & Bloom,

2012). While both fasting and feeding influence the circulating level of AgRP, increased AgRP has also been shown to occur in times of high-energy requirement - such as during pregnancy and lactation (Breen, Conwell & Wardlaw, 2005; Sorensen et al., 2002). This further establishes the role AgRP may play in EB regulation and introduces the need to investigate the AgRP response to exercise-induced negative EB. Finally, AgRP independently influences EE by reducing oxygen consumption, suppressing thyrotropin-releasing hormone (TRH) and reducing EE from brown adipose tissue (Small et al., 2001; Small et al., 2003).

In terms of sexual differentiation, females may have higher levels of AgRP when compared to males (Kim et al., 2005; Voisey, Imbeault, Hutley, Prins & vanDall, 2002). Changes in the expression of AgRP have been shown to correspond to changes in the menstrual cycle, which presents one potential explanation as to why AgRP expression may differ between males and females (Olofsson, Pierce & Xu, 2009).

### **Neuropeptides: Anorexigenic**

**Pro-opiomelanocortin (POMC) and alpha-melanocyte stimulating hormone (alpha-MSH).** POMC is an anorexigenic neuropeptide synthesized in POMC/CART neurons of the ARC and the nucleus tractus solitaries of the brainstem (Millington, 2007; Parker & Bloom, 2012). It is the precursor protein for melanocyte-stimulating hormones, amongst other signaling molecules and plays an important role in inhibiting feeding behaviors (Millington, 2007; Yu & Kim, 2012). POMC expression is regulated by nutritional status with increased POMC mRNA expression when over-fed and decreased expression when energy-restricted (Hagan et al., 1999; Mizuno et al., 1998). Alpha-MSH is a neuropeptide expressed in the lateral ARC which signals downstream by binding to

MC3R and MC4R receptors (Stanley et al., 2005). Alpha-MSH action in the ARC suppresses appetite and has also been suggested to increase EE through various mechanisms, including: increased oxygen consumption and activation of the thyroid axis, brown adipose, and the sympathetic nervous system (Huszar et al., 1997; Kim et al., 2000; Pierroz et al., 2002; Valaasi et al., 2008; Yasuda, Masaki, Kakuma, & Yoshimatsu, 2004). The activation of MC4R receptors may elicit distinct responses depending upon the area of the brain that is activated. Alpha-MSH is known to elicit a negative EB, however, to the author's knowledge no research has compared POMC/alpha-MSH expression between males and females.

**Cocaine and amphetamine-regulated transcript (CART).** CART is co-localized with POMC neurons in the hypothalamic ARC (Cowley et al., 2001). Current research indicates that there may be various populations of CART-expressing neurons that play distinctive roles in ER behavior (Stanley et al., 2005). Preliminary studies on mice have shown that central injection of CART decreases food intake (Lambert et al., 1998). However, more recent research has found that when CART is injected into hypothalamic nuclei (ARC and VMN), EI actually increases (Abbott et al., 2001). Additionally, food restriction that yields a negative EB reduces CART expression in the ARC, however, peripheral replacement of leptin can stimulate CART expression (Kristensen et al., 1998). Currently, the mechanisms by which CART influences ER are poorly understood and there is no research to date that has investigated potential sex differences in CART expression.

EI and EE maintain energy homeostasis and are regulated by complex interconnections between hypothalamic nuclei and their endocrine signals, in addition to psychological motivators for eating behavior (Schwartz et al., 2000; Stanley et al., 2005).

It is largely unknown how, or if this pathway's interconnections and signals differ between males and females.

### **Sex Differences in EI/EE Regulation**

Although sex differences in factors that regulate EB have been observed, the specific mechanisms are largely unknown. It has been suggested that the distribution of adipose mass, largely influenced by sex steroids (i.e. estrogen, androgens), is involved in the sex-based variation seen in energy homeostasis and substrate metabolism (Cortright & Koves, 2000). A combination of the direct effects of sex hormones on bone and lean tissue, as well as indirect effects of sex hormones on the hypothalamus, may be responsible for the ratio of fat mass to fat-free mass (Tsatsoulis, Wycoff & Brown, 2009). Hypothalamic expression of NPY is effected differentially by androgens and estrogen (Clegg, Brown, Woods & Benoit, 2006). Also, the sex steroid estrogen, which reduces visceral fat in both sexes, may influence adipose distribution by means of modulating leptin sensitivity and, in females, may reduce EI in response to central leptin administration more so than males (Clegg et al., 2006). Additionally, absolute EE is suggested to be lower in women compared to men (Tooze et al., 2007; Westerterp & Elbers, 1999). This is a result of smaller body size and higher ratio of fat mass to fat-free mass compared to men; both which are strong predictors of EE (Lovejoy, Sainsbury, & the Stock Conference 2008 Working Group, 2008). These sex-based discrepancies in EE may be exacerbated in response to PA.

Regular exercise is associated with decreased adipose mass in men; however, this relationship is not as consistently observed in women (Paul, Novotny & Rumpler, 2004; Westerterp & Goran, 1997). The essential fat (EF) mass in females is 12%, four times

greater than males, which accounts for approximately 4% of total body weight (Comitato, Saba, Turrini, Arganini & Virgili, 2015). Reducing body fat levels below the EF level, as may be seen with strenuous exercise and dieting, can significantly impair overall health with critical implications on women's health (Westerterp & Goran, 1997). EF is crucial for hormone-related functions, childbearing and normal physiological functioning (Westerterp, Meijer, Janssen & ten Hoor, 1992; Westerterp & Goran, 1997). The protection of EF for reproductive purposes is one suggestion as to why females may see less fat loss from exercise when compared to men. Additionally, women also carry approximately 5-9% of their total body fat as "sex-specific fat", distributed between the breasts, lower body subcutaneous fat, and genital regions (Kissebah & Krakower, 1994). It is possible that sex-specific fat is more resistant to oxidation for childbearing and hormonal-functioning purposes, further enhancing resistance for fat-loss from exercise in women.

In order to protect EF levels and maintain reproductive health, it has been suggested that women may be more likely than men to increase EI following exercise as a means to compensate for increased EE (Stubbs et al., 2002a; Westerterp & Goran, 1997). Research in this area has found increased EI in women, but not in men, following identical exercise protocols (McLaughlin, Malkova, & Nimmo, 2006; Staten, 1991; Stubbs et al., 2002a; Stubbs et al., 2002b). Although the specific mechanisms for these sex differences in compensatory EI are unknown, it appears that estrogen levels impact EI and EE in women. It has been suggested that females with lower circulating estrogen levels, compared to those women with higher estrogen, tend to increase meal-time EI and also have significantly reduced 24-h and physical activity EE (Asarian & Geary, 2002; Clegg et al., 2006; Lovejoy et al., 2008). Increased expression of orexigenic

neuropeptides NPY and AgRP, and decreased expression of anorexigenic POMC may be responsible for elevated EI seen in women with lower estrogen levels (Sainsbury, Cooney & Herzog, 2002). Additionally, lack of estrogenic activity in the hypothalamus may be responsible for the lower EE measured in women when estrogen levels are decreased with the natural fluctuations that occur during the menstrual cycle (Clegg et al., 2006; Pelletier et al., 2007; Sainsbury et al., 2002). As energy homeostasis and appetite regulation have shown some variation between sexes, and have both clinical and physiological relevance, more research needs to be completed in order to fully understand the impact of sex on the response of ER hormones to exercise.

### **EE and EI Fluctuations Across the Menstrual Cycle**

Energy intake fluctuates throughout the menstrual cycle and is increased in women around the time of menses when estrogen levels are low (Barr, Janelle & Prior, 1995; Bisdee et al., 1989; Buffenstein et al., 1995; Dye & Blundell, 1997). Fluctuations in eating habits have also been seen across the menstrual cycle, where EI is lower during the follicular phase compared to the luteal phase, but lowest around the time of ovulation when levels of circulating estrogen peak (Asarian & Geary, 2002; Lovejoy et al., 2008). These cyclical changes in EI have been mimicked with estradiol replacement but not progesterone replacement, suggesting that the variation in circulating levels of estrogen is responsible for cyclical variations in EI throughout the menstrual cycle (Asarian & Geary, 2002). Some studies have also shown measurable variation in EE over the course of the menstrual cycle, though the majority of research done in this area shows that women remain in EB from month-to-month (Davidsen, Vistisen & Astrup, 2007). In light of this

information, controlling for potential fluctuations in EB throughout the menstrual phase appears to be critical.

### **Acute Exercise on Appetite Regulation**

Given the interest in the use of exercise for weight loss or weight maintenance, there has been more research examining the effect of exercise on the endocrine signals that regulate appetite and EB. Currently there are many conflicting results, likely due to differences in duration, intensity, and type of exercise performed. Recent studies have begun investigating the response of appetite regulating hormones following manipulation of these variables (exercise intensity, type and duration). Here, intensity and duration are of particular interest. Both moderate-intensity continuous endurance training (END), a form of aerobic exercise (AE) performed between 50-70%  $VO_{2max}$ , and high-intensity interval training (HIIT), repeated maximal effort exercise bouts separated by lower effort activity or rest, may elicit comparable effects on body composition, occurring over a reduced training duration with HIIT (Fisher et al., 2015; MacPherson, Hazell, Olver, Paterson, & Lemon, 2011). This finding may provide insight into newer, more desirable training techniques that deliver comparable health benefit with less time commitment. Some believe that HIIT is a poor exercise prescription for previously sedentary individuals as the extreme intensity is a deterrent, decreasing ones' self-esteem and motivation and finally decreasing exercise affect (Biddle & Batterham, 2015; Hardcastle, Ray, Deale & Hagger, 2014). On the contrary, HIIT has been found to be more enjoyable than END; increasing motivation due to the improvements observed in body composition and body mass in a reduced time demand, outweighing the aversive effects of intensity (Del Vecchio, Gentil, Coswig & Fukuda, 2015; Jung, Little & Batterham, 2015). The

effect of exercise-induced EE on appetite regulating hormones and EI following exercise are also of interest when studying EB and the connection between EE and EI on body weight regulation. Examination on the effect of exercise intensity and duration on each of the ER neuroendocrine signals discussed earlier is to follow (Hazell et al., 2016; Schubert, Desbrow, Sabapathy & Leveritt, 2013; Schubert, Sabapathy, Leveritt & Desbrow, 2014).

### **Acute Exercise and Orexigenic Hormones**

**Ghrelin.** To investigate the effect of increased EE on subsequent appetite related hormonal activity, research has examined the effect of acute exercise on plasma acylated ghrelin concentration. Varying results have been observed and an ultimate effect of exercise on circulating ghrelin concentrations has not been determined. Numerous studies have examined the effect of exercise on ghrelin and the response varies depending on the intensity and duration of the exercise. No change in the concentration of plasma acylated ghrelin was found in response to low intensity, steady state END activity at 50-70% of  $VO_{2max}$  (Balaguera-Cortes, Wallman, Fairchild, & Guelfi, 2011; Hagobian et al., 2013; King et al., 2011a; Larson-Meyer et al., 2012; Sim, Wallman, Fairchild & Guelfi, 2013). Additionally, END exercise at a moderate, continuous intensity (50-75%  $VO_{2max}$ ), 30-90-min in duration commonly elicited reduced concentrations of acylated ghrelin by up to ~30-40% in adult populations (Broom, Stensel, Bishop, Burns, & Miyashita, 2007; Broom, Batterham, King & Stensel, 2009; Deighton, Karra, Batterham & Stensel, 2013b; Kawano et al., 2013; King, Wasse, Broom & Stensel, 2010a; Sim et al., 2013; Tiryaki-Sonmez et al., 2013; Wasse, Sunderland, King, Miyashita & Stensel, 2013). This suggests that the intensity of exercise influences ghrelin's response. Several researchers

have also suggested that an exercise-induced diversion of splanchnic blood flow causing suppressed ghrelin release from the gut could be a reason that acylated ghrelin levels, and appetite, are decreased during and after exercise (Broom et al., 2007; Deighton et al., 2013; Hazell, Islam, Townsend, Schmale, & Copeland, 2016; King et al., 2011a; Larson-Meyer et al., 2012). The above results indicate that AE at a moderate intensity may not consistently provide a strong enough physiological stimulus to reduce plasma acylated ghrelin concentration, however there is some likelihood that reductions in plasma acylated ghrelin could occur - perhaps dependent upon the duration and mode of exercise and the population studied.

HIIT, in comparison, has been shown to elicit a more consistent suppression of ghrelin release from the gut resulting in lower concentrations of circulating plasma acylated ghrelin. Studies of END, HIIT and sprint interval training (SIT) (a form of HIIT defined as repeated 30-sec maximal effort bouts separated by 4-min of active recovery) cycling have reported decreases of acylated ghrelin consistently in HIIT and SIT groups only (Deighton, Barry, Connon & Stensel, 2013a; Sim et al., 2013). Studies of END swimming and cycling have measured suppressed acylated ghrelin following exercise bouts, though to a smaller magnitude and less consistently than exercise at high intensity (>75%  $VO_{2max}$ ) (Kawano et al., 2013; King, Wasse & Stensel, 2011b). Further demonstrating the effect of intensity on acylated ghrelin concentration, SIT studies of six maximal effort 30-s sprints, separated by 4-min rest, have shown both immediate and prolonged (45-min post training) suppression of acylated ghrelin (Deighton et al., 2013a). Decreases in acylated ghrelin averaged >50% during exercise, which was significantly greater than that recorded during END at a longer duration (60-min). This could be due to a decrease in splanchnic blood flow and increased SNS activity, which occurs to a

greater degree during HIIT exercise (Clausen, 1977; Rowell, 1974). This prolonged suppression of acylated ghrelin may be responsible for promoting a sustained negative EB and also the reported effect on decreased body fat comparable to that of endurance training. This warrants further investigation.

### **Acute Exercise and Anorexigenic Hormones**

**PYY.** Investigation into the acute response of plasma PYY concentration to END running and cycling has shown elevated levels of plasma PYY concentrations (Table 1.1) by up to 25-60% from resting values during, and up to 24-h into recovery from exercise (Broom et al., 2009; Cooper et al., 2011; Deighton et al., 2013a; Deighton et al., 2013b; Hagobian et al., 2013; Kawano et al., 2013; Larson-Meyer et al., 2012; Russell et al., 2009; Ueda et al., 2009a; Ueda, Yoshikawa, Katsura, Usui & Fujimoto, 2009b). Studies examining the acute effect of AE on circulating PYY levels have included low-to moderate-intensity (30-65%  $VO_{2max}$ ) END running, walking and cycling sessions, 30-90 min in duration. Increases in circulating PYY concentration were found in response to a wide variety of exercise intensities (45-75% of  $VO_{2max}$ ), and durations (30-60-min), as shown in in Table 1.1. Moreover, higher-intensity (>70%  $VO_{2max}$ ) cycling caused a greater increase in PYY from baseline compared to lower-intensity exercise (Ueda et al., 2009b). Additionally, an increase in PYY following time and intensity-matched running and walking (60-min at 70%  $VO_{2max}$ ), was also reported in habitual walkers and endurance trained female runners (Larson-Meyer et al., 2012). The difference reported here was that PYY concentration was more elevated and remained elevated for longer following running, peaking immediately following running and gradually returning to baseline 120-min into recovery. While PYY concentration peaked 30-min following

walking, it occurred to a lesser extent than after running and returned to baseline within 90-min post-exercise, suggesting that exercise type may influence PYY response (Larson-Meyer et al., 2012).

The mechanism by which PYY responds to exercise is not well understood (Hazell et al. 2016). While some studies have reported prolonged increases in PYY resulting from higher intensities of AE, others have suggested that it is not the intensity that dictates an elevated PYY response but rather the mechanism of exercise, with apparent reductions in the anorexigenic peptide in runners but not walkers (Larson-Meyer et al., 2012). It is also suggested that PYY may be more sensitive to exercise in general compared to other gut hormones, eliciting increases at lower intensities (Larson-Meyer et al., 2012). As an exception to the increases reported earlier, moderate intensity AE had no effect on circulating plasma PYY concentrations (Balaguera-Cortes et al., 2013; Cheng, Bushnell, Cannon & Kern, 2009; Hagobian et al., 2013; Holmstrup, Fairchild, Keslacy, Weinstock & Kanaley, 2013; King et al., 2011a; Sim et al., 2013). These studies observed both normal weight and obese males in both running and cycling trials. Differences observed may have been a result of energy status (fed or fasted) as PYY concentration is known to fluctuate with EB and the majority of studies that reported increased PYY concentration occurred when exercise was performed in a fed state, suggesting the response may have been due to feeding rather than exercise. In addition, different forms of PYY (active form vs. total) may have been measured which could have influenced the result obtained. The contrasting results observed exemplify the need for further investigation of the acute effect of exercise intensity on PYY. To the author's knowledge, no studies have found a decrease in PYY in response to exercise.

There have been fewer studies of high-intensity exercise on PYY concentration. Studies to date have shown either transient increases in concentration (Beaulieu, Olver, Abbott & Lemon, 2015; Deighton et al., 2013b; Kawano et al., 2013; Martins, Morgan, Bloom & Robertson, 2007) or no change in plasma PYY levels at all in response to HIIT cycling (Deighton et al., 2013b; Sim et al., 2013). SIT yielded no change in PYY concentration in normal weight males after completing three-min of maximal effort work (6 x 30-sec maximal effort bouts, separated by 4-min rest) (Deighton et al., 2013a). Similarly, SIT cycling in overweight males elicited no change in PYY concentration (Sim et al., 2013), however a short-lived (<1-h) increase in PYY concentration has also been observed (Beaulieu et al., 2014). This dissimilarity may be due to the comparison of overweight and normal weight participants and measurement of different forms of PYY. Additionally, 40-min of END cycling (10x: 4-min bouts, separated by 2 min rest to elicit 85%  $VO_{2max}$ ) yielded increases in PYY concentration greater than observed after 60-min of moderate intensity AE in men (Deighton et al., 2013b). Taken together, these results indicate that intensity may have important implications on the exercise-induced increase of plasma PYY, though the exact mechanisms are unknown and warrant further examination.

**PP.** Few studies have investigated the acute effect of exercise on plasma PP concentrations, nevertheless, PP seems to increase after exercise (Table 1.1). The reported time period with which these increases have occurred varies widely (during exercise vs. during recovery) amongst studies, as does the magnitude of the response. Increased circulating plasma PP measurements were observed during recovery following 180-min of low intensity END cycling (Hilsted et al., 1980), 45-min of moderate intensity END running (Balaguera-Cortes et al., 2011) and 45-min low intensity cycling

(Greenberg, Marliss & Zinamn, 1986). It seems as though exercise at a high intensity (>75% VO<sub>2max</sub>) induces a faster response, by which increases in circulating PP concentration occur shortly after cessation of exercise (Martins et al., 2007). Studies that measured PP throughout the exercise bout but not during post-exercise recovery did not show changes in PP concentrations (Sim et al., 2013). Additionally, elevated PP has been observed up to 2-h following graded running to exhaustion, further demonstrating that the exercise-induced response of PP is delayed (Balaguera-Cortes et al., 2011; Hilsted et al., 1980; Martins et al., 2007). While it is known that exercise results in increased PP concentrations up to 2-h into recovery from an exercise bout, the influence of intensity on this prolonged and delayed response is not well known.

**GLP-1.** Little research has been completed investigating the acute effect of exercise intensity on plasma GLP-1 concentrations and EI regulation. Of the available literature, there is a balance of studies that report the exercise-induced response of total GLP-1 concentration to those who reported the response of active GLP-1 concentration, and additional studies that did not identify the form of GLP-1 measured. The variance in the form of GLP-1 measured is likely to influence on the results that are obtained. Three studies shown in Table 1.1 reported substantial increases in GLP-1<sub>9-36</sub> during, immediately after, and 90-min into recovery from AE ranging from 30-60 min (Larson-Meyer et al., 2012; Ueda et al., 2009a, 2009b). These increases range anywhere from 25-50% above baseline concentrations. Increases in GLP-1<sub>9-36</sub> concentration have also been reported following longer AE running, including 120-min and marathon durations (O'Conner, Johnston, Buchanan, Boreham, Trinick, & Riddoch, 1995). No effect on plasma GLP-1 concentration was seen in response to 30-min of END cycling (Kawano et al., 2013). However, END cycling at greater volume, lower intensity (duration = 51-min,

60% HR<sub>max</sub>) and estimated greater caloric expenditure caused an increase in plasma GLP-1 concentrations (Martins et al., 2007). Additionally, the effect of 60-min of treadmill walking in untrained females compared with 60-min of END running in trained females, with an estimated higher caloric expenditure in the running group, found that the trained females saw a resultant increase in plasma GLP-1 concentration that was not found in the group of untrained walkers (Larson-Meyer et al., 2012). Exercise may influence GLP-1 concentration through reduced gastric motility, increased release of interleukin-6 with skeletal muscle contraction, and elevated blood glucose and insulin following exercise, stimulating the release of GLP-1 which has a known role as an incretin hormone (Hazell et al., 2016). The current understanding of the GLP-1 response to exercise suggest that caloric expenditure and exercise duration, or possibly training status, rather than intensity may be more critical to exercise-mediated release of GLP-1.

**Leptin.** Examination of the effect of acute exercise on plasma leptin levels has resulted in observations of either decreased plasma leptin concentration or no change in concentration at all. Generally, low and moderate intensity AE of varying duration (30 to 120-min) has no effect on plasma leptin concentrations (Balaguera-Cortes et al., 2011; Cheng et al., 2009; Ferguson et al., 2004; Jurimae et al., 2006; Jurimae et al., 2007a; Kyriazis et al., 2007; Landt et al., 1997; Racette, Coppack, Landy & Klein, 1997; Sim et al., 2013; Torjman, Zafeirdis, Paolone, Wilkerson & Considine, 1999; Vantansever-Ozen, Tiryaki-Sonmez, Bugdayci & Ozen, 2011). Additionally, exercise intensity had no influence on serum leptin levels during, or into recovery from, endurance AE, though the duration of activity may have influenced concentration (Weltman et al., 2000). Reductions in circulating leptin by up to 18% have been observed up to 24-h after completion of 60-min END running, with reductions more than doubling that after 48-h

of recovery (Olive & Miller, 2001). As leptin is known to fluctuate with circadian rhythm, it may be important to control for this over the course of an exercise trial to limit confounding effects (Kalsbeek et al., 2000). Numerous studies, as shown in Table 1.1, have found exercise-induced reductions in leptin concentration following long-duration bouts of: cycling, swimming and running (>2-h). This includes two studies that investigated plasma leptin concentration during and after marathon and ultra marathon distances (Landt et al., 1997; Leal-Cerro et al., 1998). Decreased leptin was found in recovery from exercise bouts of varying EE but not during the actual sessions, further emphasizing that any reductions in leptin resulting from exercise are delayed and do not occur immediately (Essig, Alderson, Ferguson, Bartoli & Durstine, 2000). Again, fluctuations of leptin that occur with circadian rhythm may be, at least in part, responsible for these prolonged reductions.

The effect of HIIT exercise on plasma leptin concentrations is unclear. No change in plasma leptin was found in response to HIIT and END cycling in overweight men when compared to baseline (Sim et al., 2013). However, END cycling produced a transient decrease in serum leptin concentrations immediately after exercise, which then reversed back to baseline levels less than 2-h into recovery in a separate male population (Fisher, Van Felt, Zinder, Landt & Kohrt, 2001). Furthermore, graded cycling and running protocols yielded no change in leptin concentrations during- or post-exercise when compared to baseline levels, even when participants performed to maximal volitional fatigue (Marzullo et al., 2008; Olive & Miller, 2001; Sliwowski, Lorens, Konturek, Bielanski & Zoladz, 2001; Torjman et al., 1999; Toshinai et al., 2007; Zoladz et al., 2005). When taken together, results of these studies provide evidence that the duration

of exercise, rather than intensity, may be responsible for exercise-induced changes in plasma leptin concentration.

### **Acute Exercise and Sex Differences in Energy Regulation**

Observation of the appetite-regulating gut hormones is well reported in healthy males but there is far less available research in female populations. Females are often overlooked as exercise research subjects because controlling for potential confounding factors related to hormonal fluctuations associated with menstruation make them a more complicated group. In studies that did differentiate between sexes, measurable differences were reported between men and women in circulating levels of ER peptide hormones leptin and ghrelin following acute exercise (Hagobian et al., 2008). Additionally, the intensity and duration of the exercise bouts in studies that utilized female participants were widely varied, making comparison to male data difficult (Hagobian et al., 2013; Larson-Meyer et al., 2012). Due to the innate physiological and morphological differences between sexes, measurement of the sex-specific hormone response to exercise provides evidence as to whether or not different approaches should be taken when developing exercise protocols for both sexes.

### **Acute Exercise, Orexigenic Hormones and Sex Differences**

**Ghrelin.** Generally speaking, results are inconclusive when comparing the response of circulating acylated ghrelin to exercise in men and women – this is summarized in Table 1.2. When male and female subjects (follicular phase) participated in identical cycling bouts at 50-56%  $VO_{2max}$ , expending ~30% of total daily energy expenditure (TDEE), no change in male acylated ghrelin concentrations was observed whereas female levels decreased significantly (Hagobian et al., 2008). This differed with

various other studies, maybe because of differing body composition, or exercise intensity and exercise duration in several of the other studies reported in Table 1.2. For example, there was no change in ghrelin concentration in normal weight females following running or walking for 60-min at 70%  $\text{VO}_{2\text{max}}$  during the follicular phase of the menstrual cycle (Larson-Meyer et al., 2012), however an increase in plasma ghrelin concentration was found in obese women cycling at 50-60%  $\text{VO}_{2\text{max}}$  (Hagobian et al., 2008) and a decrease observed in ghrelin concentration following END graded cycling (Marzullo et al., 2008). As phase of menstrual cycle was not noted in all studies, it is possible that differences in the phase of menstrual cycle may have influenced the differing of results.

### **Acute Exercise, Anorexigenic Hormones and Sex Differences**

**PYY.** Acute exercise has caused either transitory increases or no significant change in circulating plasma concentration in females (Hagobian et al., 2013; Larson-Meyer et al., 2012). This is comparable to what is observed in males, suggesting there is no significant sex difference (Hagobian et al., 2013; Holmstrup et al., 2013; Larson-Meyer et al., 2012; Martins et al., 2007; Russel et al., 2009). Refer to Table 1.2 for detailed findings.

**PP.** Currently, there is limited literature available that presents the relationship between plasma PP and acute exercise in both men and women. As illustrated in Table 1.2, three studies have examined exercise-induced changes in PP in both male and female participants. Increases in plasma PP occurred in each, though results of males and females were not separated (Martins et al., 2007; O'Conner et al., 1995). Past research has indicated that there are innate physiological differences between males and females that may result in differences in the release of ER hormones, including PP, in response to

acute exercise (Hagobian et al., 2008). As such limited data are available, and the potential for these sex differences has been suggested, further research that investigates PP concentrations following acute exercise in females is needed.

**GLP-1.** Limited literature is available that examines the acute effect of exercise intensity on plasma GLP-1 concentration in females. When comparing studies that have included females, the response appears to be comparable to males. Female participants displayed transient increases in GLP-1 concentrations in response to time matched bouts of continuous END running at 70%  $VO_{2max}$  and END cycling at 60%  $HR_{max}$  (Larson-Meyer et al., 2012; Martins et al., 2007). Exceptions to this were reported in bouts of continuous END walking at 70% of  $VO_{2max}$  that yielded no change in plasma GLP-1 concentration (Larson-Meyer et al., 2012) and walking at 70-75%  $VO_{2max}$ , which caused decreased GLP-1 concentration during recovery (Unick et al., 2010). As the intensity of exercise performed was consistent between studies, this difference in GLP-1 response suggests that the mode of exercise may have an influence on GLP-1 concentration post-exercise. Overall, the majority of research shows an increase in plasma GLP-1 concentration in both men and women in response to acute exercise. Additionally, the lack of available data in the female population reduces the understanding of ER in an entire population and presents a wide gap in ER research.

**Leptin.** Again, limited literature is available that measures plasma leptin concentration in response acute exercise in females. Most available data shows no sex difference in leptin concentration in response to exercise (Ferguson et al., 2004; Hickey et al., 1996; Landt et al., 1997; Marzullo et al., 2008). The studies that revealed no sex difference in leptin response all reported results in “healthy adults”, not in males and females independently. When the response in men and women was separated, there was

still no change in leptin concentration following low intensity aerobic cycling in men, however a decrease in leptin concentration was observed in females (Hagobian et al., 2008). By combining data from men and women it becomes impossible to identify specific dietary and exercise specific recommendations for women.

### **Summary**

Exercise-induced decreases of acylated ghrelin and increases of PYY, PP and GLP-1 are likely responsible, in part, for the short-term negative EB that can result from an acute exercise session. Exercise intensity appears to influence the decrease of acylated ghrelin and increase of PYY, with greater decreases resulting from high-intensity compared to moderate and low intensity exercise. Additionally, GLP-1 and PYY appear to be affected more by longer duration exercise, suggesting that a threshold of EE may need to be surpassed for increased release of these hormones. Exercise-induced secretion of PP shows a delayed and prolonged response, with higher intensity exercise producing faster and more prolonged release when compared to moderate and low intensity bouts. It appears that high-intensity exercise protocols elicit a neuroendocrine response that could result in increased satiation and decreased appetite compared to lower intensity exercise.

Preliminary research examining the response of ER hormones to acute exercise, coupled with our understanding of the innate differences in EB requirements between men and women, reveals the potential for sex-differences in the exercise-induced response of appetite-regulating hormones. In summary, greater decreases in acylated ghrelin appear to occur in females compared to males at matched intensity and duration. In contrast, gut hormones PYY, PP, GLP-1 and leptin appear to respond the same to duration- and intensity-matched exercise bouts in both sexes. With such limited data

available as to the way that females respond to exercise, and the innate physiological and endocrinological differences between sexes, research that focuses on a female population is needed. Generalizing conclusions from a male population to female population is problematic and female specific research is needed. Exercise research including females will provide important information for women's health, including evidence based knowledge for the prescription of specific and effective diet and exercise protocols.

### **Hypothesis and Purpose**

While interest in the potential appetite suppressing effects of exercise continues, experimental evidence to illustrate the effect of exercise intensity on appetite regulating hormones is limited. Even more limited is examination of the effect of exercise, of any mode and intensity, on appetite and appetite regulating hormones in females. In women, the influence of exercise-induced appetite suppression and its contribution to creating a negative EB, with the potential for eliciting fat loss, remains largely undocumented by scientific research. Studies on the effect of cardiovascular and resistance exercise on the ER hormones in men have begun only recently. With the known physiological and endocrinological differences between sexes there is a need for evidence-based information that has examined the sex-dependent physiological effect of exercise on appetite hormones and ER.

The purpose of this thesis was to examine the effect of exercise intensity on satiety hormones PYY and GLP-1 in women and to then examine whether any sex differences exist in the response of these hormones and subjective hunger to moderate-intensity END cycling. Modest increases in both PYY and GLP-1 were expected in women immediately following exercise, with greater increases in satiety hormones

anticipated following SIT and only brief increases, returning to baseline by 90-min into recovery with END. Increases in perceptions of hunger were expected in men and women in recovery from END cycling, with the expectation that SIT would result in satiety immediately post-exercise and into recovery from exercise. We expected that increases in both GLP-1 and PYY would be greater in men compared to women, with increased satiety associated with increased levels of these hormones in men.

Following this review of available scientific literature on the effect of exercise on appetite regulating hormones, particularly as it relates to how exercise at varying intensity may differentially affect men and women, the experimental research conducted as part of the thesis project will be presented in two separate chapters. Chapter two presents the comparison of END and SIT on appetite regulating hormones in women. Next, chapter three presents the sex comparison on the effect of END on satiety hormones GLP-1 and PYY. Finally, to conclude, a general discussion will summarize the result of the studies as a whole.

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**Table 1.1: Studies investigating the acute effect of exercise on peripheral hormones involved in energy regulation.**

References	Subjects	Fed	Exercise Mode, Volume, Intensity, Duration	Leptin	Total Ghrelin	Ghrelin	PYY	GLP-1	PP
Balaguera-Cortes et al. 2011	NW men	N	AE running (70%VO <sub>2max</sub> , 45 min)	↔	∅	↔	↔	∅	
Bilski et al., 2013	NW men	N	AE cycling (30% VO <sub>2max</sub> , 30min) HIIT (firefighter fitness test: running 6 min, 9kph; Pull hammer 20X: pull force 25 kG; Ladder: 30m vertical; Cycle: 200W 1 min; Smoke chamber and maze: ~5min	∅	↑	∅	∅	∅	∅
Broom et al., 2009	NW men	N	AE running (70% VO <sub>2max</sub> , 60 min)	∅	∅	↓	↑	∅	∅
Boom et al., 2007	NW men	N	AE running (75% VO <sub>2max</sub> , 60 min)	∅	∅	↓	∅	∅	∅
Burns et al, 2007	NW men	N	AE running (75% VO <sub>2max</sub> , 60 min)	∅	↔	∅	∅	∅	∅
	NW women	N	AE running (75% VO <sub>2max</sub> , 60 min)	∅	↔	∅	∅	∅	∅
Cheng et al., 2009	NM men	N	AE cycling (60% VO <sub>2max</sub> , 50 min)	↔	↑	∅	↔	∅	∅
		Y	AE cycling (60% VO <sub>2max</sub> , 50 min)	↔	↔	∅	↔	∅	∅
Christ et al, 2006	NM men	Y	AE cycling (50% W <sub>max</sub> , 180 min)	↓	↑	∅	∅	∅	∅
Cooper et al., 2011	NW men	Y	AE cycling (45% VO <sub>2max</sub> , 60min)	↓	↔	∅	↑	∅	∅
Dall et al., 2002	NW men	N	AE cycling (100% LT, 45 min)	∅	↔	∅	∅	∅	∅
Deighton et al., 2013a	NW men	Y	AE cycling (68% VO <sub>2max</sub> , 60 min) SIT cycling (6X: 30-s Wingate, 4 min rest)	∅	∅	↓	↑	∅	∅
				∅	∅	↓	↔	∅	∅
Deighton et al, 2013b	NW men	Y	AE cycling (60% VO <sub>2max</sub> , 60 min) MIT cycling (10X: 4 min 85% VO <sub>2max</sub> , 2 min rest)	∅	∅	∅	↑	∅	∅
				∅	∅	∅	↑	∅	∅
Duclos et al., 1999	NW men	Y	AE running (65-75% VO <sub>2max</sub> , 120 min)	↓	∅	∅	∅	∅	∅
Erdmann et al., 2007	NW adults		AE cycling (50W & 100W, 30min) AE cycling 50W, 30, 60 & 120 min)	∅	∅	↔	∅	∅	∅
				∅	∅	↑	∅	∅	∅
Essig et al., 2000	NW men	Y	AE running (70% VO <sub>2max</sub> , 800kcal)	↓	∅	∅	∅	∅	∅

			AE running (70% VO <sub>2max</sub> , 1500 kcal)	↓	∅	∅	∅	∅	∅
Ferguson et al., 2004	NW adults		AE cycling (65% VO <sub>2max</sub> , 60min)	↔	∅	∅	∅	∅	∅
Fisher et al., 2001	NW men	Y	MIT cycling (4X: 5 min 85% VO <sub>2max</sub> , 3 min 50% VO <sub>2max</sub> )	↑/↓	∅	∅	∅	∅	∅
Greenberg et al., 1986	NW adults	Y	AE cycling (50% VO <sub>2max</sub> , 45 min)	∅	∅	∅	∅	∅	↑
Hagobian et al., 2013	NW men	N	AE cycling (70% VO <sub>2max</sub> , until 30% TDEE)	∅	∅	↔	↔	∅	∅
	NW women	N	AE cycling (70% VO <sub>2max</sub> , until 30% TDEE)	∅	∅	↔	↑	∅	∅
Hickey et al., 1996	NW adults	Y	AE running (20 miles total, intensity unknown)	↔	∅	∅	∅	∅	∅
Hilsted et al., 1980	NW men	N	AE cycling (40% VO <sub>2max</sub> , 180 min)	∅	∅	∅	∅	∅	↑
Holmstrup et al., 2013	OB adults	Y	AE (60-65% VO <sub>2max</sub> , 60 min)	∅	∅	∅	↔	∅	∅
			Intermittent ex (12X: 5 min @ 60-65%, 55 min rest)	∅	∅	∅	↔	∅	∅
Jensen et al., 1994	NW men	N	AE cycling (75% VO <sub>2max</sub> , 15 min)	∅	∅	∅	∅	∅	∅
Jurimae et al., 2007	NM men	Y	AE rowing (5bpm < AT, 30 min)	↔	↔	∅	∅	∅	∅
			AE rowing (5 bpm > AT, 30 min)	↔	↔	∅	∅	∅	∅
Jurimae et al., 2007 b	NW men	Y	AE rowing (maximal 6000 m test)	↓	↑	∅	∅	∅	∅
Jurimae et al., 2006	NW men		AE rowing (6.5km at AT)	↔	∅	∅	∅	∅	∅
Jurimae et al., 2009	NW men		AE rowing (2 hours)	↓	↑	∅	∅	∅	∅
Karamouzis et al., 2002	NW men	Y	AE 25-km swim race (6.9-10.5 hr)	↓	∅	∅	∅	∅	∅
Kawano et al., 2013	NW men	N	MIT cycling (3X: 10 min 64% VO <sub>2max</sub> , 5 min rest)	∅	∅	↓	↑	↔	∅
King et al., 2010a	NW men	N	AE running (70% VO <sub>2max</sub> , 90 min)	∅	∅	↓	∅	∅	∅
King et al., 2010b	NW men	N	AE walking (45% VO <sub>2max</sub> , 60 min)	∅	∅	↔	∅	∅	∅
King et al., 2011a	NW men	N	AE running (70% VO <sub>2max</sub> , 90 min)	∅	∅	↔	↔	∅	∅
King et al., 2011b	NW men	Y	MIT swimming (6X: 7 min, 3 min rest)	∅	∅	↓	∅	∅	∅

Kraemer et al., 2004	NW men	N	GXT running (60% VO <sub>2max</sub> , 10 min + 75% VO <sub>2max</sub> , 10 min + 90% VO <sub>2max</sub> , 5 min + 100% VO <sub>2max</sub> , 2 min; 3.5-4 min rest)	∅	↔	∅	∅	∅	∅
Kyriazis et al., 2007	OW males		AE exercise (58.4% VO <sub>2max</sub> , 60 min)	↔	↔	∅	∅	∅	∅
Landt et al., 1997	NW adults	N	AE running (75% VO <sub>2max</sub> , 120 min)	↔	∅	∅	∅	∅	∅
	NW men	Y	AE running (101-mile ultramarathon)	↓	∅	∅	∅	∅	∅
Larson-Meyer et al., 2012	NW women	Y	AE running (70% VO <sub>2max</sub> , 60 min)	∅	↔	↔	↑	↑	∅
			AE walking (70% VO <sub>2max</sub> , 60 min)	∅	↔	↔	↑	↔	∅
Leal-Cerro et al., 1998	NW men	Y	AE running(marathon race)	↓	∅	∅	∅	∅	∅
Mackelvie et al., 2007	NW/OW men	Y	AE cycling (65% HR <sub>max</sub> , 60min)	∅	↔	∅	↑	↑	↑
Martins et al., 2007	NW adults	Y	MIT cycling (3X: 60% HR <sub>max</sub> , 19 min, 3 min rest)	∅	↔	∅	↑	↑	↑
Marzullo et al., 2008	NW adults	N	GXT cycling (20W + 20W min-1 to exhaustion)	↔	↔	↓	∅	∅	∅
O'Conner et al., 1995	NW adults	Y	AE running (marathon)	∅	∅	∅	∅	↑	↑
O'Conner et al., 1995	NW men		AE running (60% VO <sub>2max</sub> , 120min)	∅	∅	∅	∅	↑	∅
Olive and Miller et al., 2001	NW men	Y	GXT running (graded VO <sub>2max</sub> test)	↔	∅	∅	∅	∅	∅
			AE running (75% VO <sub>2max</sub> , 60 min)	↓	∅	∅	∅	∅	∅
Racette et al., 1997	NW men	N	AE cycling (50% VO <sub>2max</sub> , 60 min)	↔	∅	∅	∅	∅	∅
	OW men	N	AE cycling (50% VO <sub>2max</sub> , 60 min)	↔	∅	∅	∅	∅	∅
Russel et al., 2009	NW adults	Y	AE running (62% VO <sub>2max</sub> , 90min + 10-km time trial)	∅	↑	∅	↑	∅	∅
Sartorio et al., 2008	NW men	N	AE cycling (80% VO <sub>2max</sub> , 60-90min)	∅	↓	∅	∅	∅	∅
	NW women	N	AE cycling (80% VO <sub>2max</sub> , 60-90min)	∅	↔	∅	∅	∅	∅
Schmidt et al., 2004	NW men	N	AE running (50% VO <sub>2max</sub> , 20 min)	∅	↔	∅	∅	∅	∅
			AE running (70% VO <sub>2max</sub> , 20 min)	∅	↔	∅	∅	∅	∅
			HIIT running (10X: 30-s 90% VO <sub>2max</sub> , 30-s rest)	∅	↔	∅	∅	∅	∅

Sim et. Al., 2013	OW men	N	AE cycling (50% VO <sub>2max</sub> , 30 min)	↔	∅	↔	↔	∅	↔
			MIT cycling (6X: 1 min 100% VO <sub>2max</sub> , 4 min 50% VO <sub>2max</sub> )	↔	∅	↔	↔	∅	↔
			SIT cycling (15X: 15-s 170% VO <sub>2max</sub> , 60s 32% VO <sub>2max</sub> )	↔	∅	↓	↔	∅	↔
Sliwowski et al., 2001	NM men	Y	GXT running (2.7 kph, 10% grade + increase grade/speed every 3 min to exhaustion)	↔	∅	∅	∅	∅	↑
			GXT running (2.7 kph, 10% grade + increase grade/speed every 3 min to exhaustion)	↔	∅	∅	∅	∅	↑
Tiryaki-Sonmez et al., 2013	OW women	N	AE running/walking (50% VO <sub>2max</sub> , 60min)	↓	∅	↓	∅	∅	∅
Torjman et al., 1999	NW men	N	AE running (50% VO <sub>2max</sub> , 60 min)	↔	∅	∅	∅	∅	∅
			GXT (3 mph, 21% grade + increase grade/speed every 3 min to exhaustion)	↔	∅	∅	∅	∅	∅
Toshinai et al., 2007	NW men	N	GXT cycling (1/2 LT, 10 min +LT, 10 min + OBLA, 10 min + OBLA-peak, 10 min)	↔	↓	∅	∅	∅	∅
Ueda et al., 2009a	NM men	Y	AE cycling (50% VO <sub>2max</sub> , 60 min)	∅	↔	∅	↑	↑	∅
	OW men	Y	AE cycling (75% VO <sub>2max</sub> , 60 min)	∅	↔	∅	↑	↑	∅
Ueda et al., 2009b	NW men	Y	AE cycling (75% VO <sub>2max</sub> , 30 min)	∅	∅	∅	↑	↑	∅
			AE cycling (50% VO <sub>2max</sub> , 30 min)	∅	∅	∅	↑	↑	∅
Vatansever-Ozen et al., 2011	NW men	N	AE running (50% VO <sub>2max</sub> , 105 min + 70% VO <sub>2max</sub> , 15min)	↔	∅	↓	∅	∅	∅
Wasse et al., 2013	NW men	N	AE running (70% VO <sub>2max</sub> , 60min)	∅	∅	↓	∅	∅	∅
			AE cycling (70% VO <sub>2max</sub> , 60min)	∅	∅	↓	∅	∅	∅
Zoladz et al., 2005	NW men	Y	GXT cycling (30W + 30W*3min-1 to exhaustion)	↔	↔	∅	∅	∅	∅
		N	GXT cycling (30W + 30W*3min-1 up to 150W)	↔	↔	∅	∅	∅	∅

*Note.* Subjects in a fed state (Fed=Y) had consumed a meal within 4-h before exercise, subjects in a fasted state (Fed=N) exercised after a >8-h fast. Normal-weight and overweight classifications were defined based on body mass index (BMI) scores, normal-weight <25 kg/m<sup>2</sup> and overweight >25kg/m<sup>2</sup>. Increases and decreases reported were statistically significant (P<0.05).

Abbreviations: AE (aerobic exercise), AT (anaerobic threshold), bpm (beats per minute), EE (energy expenditure), GLP-1 (glucagon-like peptide-1). GXT (graded exercise training), HIIT (high-intensity interval training), HR<sub>max</sub> (maximum heart rate), LT (lactate threshold), MIT (moderate-intensity interval training), NW (normal-weight), OBLA (onset of blood lactate accumulation), OW (overweight), PP (pancreatic polypeptide), PYY (peptide tyrosine-tyrosine), ↔ (no change), ↓ (concentration decrease), ↑ (concentration increase), ∅ (hormone not measured)

**Table 1.2: Studies with mixed-sex samples examining the effects of exercise on peripheral hormones involved in energy regulation.**

References	Subjects	Fed	Exercise Mode, Volume, Intensity, Duration	Leptin	Total Ghrelin	Ghrelin	PYY	GLP-1	PP
Burns et al, 2007	NW men	N	AE running (75% VO <sub>2max</sub> , 60 min)	∅	↔	∅	∅	∅	∅
	NW women	N	AE running (75% VO <sub>2max</sub> , 60 min)	∅	↔	∅	∅	∅	∅
Erdmann et al., 2007	NW adults		AE cycling (50W & 100W, 30min)	∅	∅	↔	∅	∅	∅
			AE cycling 50W, 30, 60 & 120 min)	∅	∅	↑	∅	∅	∅
Ferguson et al., 2004	NW adults		AE cycling (65% VO <sub>2max</sub> , 60min)	↔	∅	∅	∅	∅	∅
Greenberg et al., 1986	NW adults	Y	AE cycling (50% VO <sub>2max</sub> , 45 min)	∅	∅	∅	∅	∅	↑
Hagobian et al., 2009	OW/OB men	Y	AE cycling (50-65%VO <sub>2max</sub> , until 30% TDEE)	↔	∅	↔	∅	∅	∅
		N	AE cycling (50-65% VO <sub>2max</sub> , until 30% TDEE)	↔	∅	↔	∅	∅	∅
	OW/OB women	Y	AE cycling (50-65% VO <sub>2max</sub> , until 30% TDEE)	↓	∅	↑	∅	∅	∅
		N	AE cycling (50-65% VO <sub>2max</sub> , until 30% TDEE)	↓	∅	↑	∅	∅	∅
Hagobian et al., 2013	NW men	N	AE cycling (70% VO <sub>2max</sub> , until 30% TDEE)	∅	∅	↔	↔	∅	∅
	NW women	N	AE cycling (70% VO <sub>2max</sub> , until 30% TDEE)	∅	∅	↔	↑	∅	∅
Hickey et al., 1996	NW adults	Y	AE running (20 miles total, intensity unknown)	↔	∅	∅	∅	∅	∅
Holmstrup et al., 2013	OB adults	Y	AE (60-65% VO <sub>2max</sub> , 60 min)	∅	∅	∅	↔	∅	∅
			Intermittent ex (12X: 5 min @ 60-65%, 55 min rest)	∅	∅	∅	↔	∅	∅
Landt et al., 1997	NW adults	N	AE running (75% VO <sub>2max</sub> , 120 min)	↔	∅	∅	∅	∅	∅
	NW men	Y	AE running (101-mile ultramarathon)	↓	∅	∅	∅	∅	∅
Larson-Meyer et al., 2012	NW women	Y	AE running (70% VO <sub>2max</sub> , 60 min)	∅	↔	↔	↑	↑	∅
			AE walking (70% VO <sub>2max</sub> , 60 min)	∅	↔	↔	↑	↔	∅
Martins et al., 2007	NW adults	Y	MIT cycling (3X: 60% HR <sub>max</sub> , 19 min, 3 min rest)	∅	↔	∅	↑	↑	↑
Marzullo et al.,	NW adults	N	GXT cycling (20W + 20W min-1 to exhaustion)	↔	↔	↓	∅	∅	∅

2008									
O'Conner et al., 1995	NW adults	Y	AE running (marathon)	∅	∅	∅	∅	↑	↑
Russel et al., 2009	NW adults	Y	AE running (62% VO <sub>2max</sub> , 90min + 10-km time trial)	∅	↑	∅	↑	∅	∅
Sartorio et al., 2008	NW men	N	AE cycling (80% VO <sub>2max</sub> , 60-90min)	∅	↓	∅	∅	∅	∅
	NW women	N	AE cycling (80% VO <sub>2max</sub> , 60-90min)	∅	↔	∅	∅	∅	∅
Tiryaki-Sonmez et al., 2013	OW women	N	AE running/walking (50% VO <sub>2max</sub> , 60min)	↓	∅	↓	∅	∅	∅
Unick et al., 2010	OW women		AE walking (70-75% HR <sub>max</sub> , 40min)	∅	∅	↓	∅	↓	∅

*Note.* Subjects in a fed state (Fed=Y) had consumed a meal within 4-h before exercise, subjects in a fasted state (Fed=N) exercised after a >8-h fast. Normal-weight and overweight classifications were defined based on body mass index (BMI) scores, normal-weight <25 kg/m<sup>2</sup> and overweight >25kg/m<sup>2</sup>. Increases and decreases reported were statistically significant (P<0.05).

Abbreviations: AE (aerobic exercise), AT (anaerobic threshold), bpm (beats per minute), EE (energy expenditure), GLP-1 (glucagon-like peptide-1). GXT (graded exercise training), HIIT (high-intensity interval training), HR<sub>max</sub> (maximum heart rate), LT (lactate threshold), MIT (moderate-intensity interval training), NW (normal-weight), OBLA (onset of blood lactate accumulation), OW (overweight), PP (pancreatic polypeptide), PYY (peptide tyrosine-tyrosine), ↔ (no change), ↓ (concentration decrease), ↑ (concentration increase), ∅ (hormone not measured)

## **Chapter 2: The effect of exercise intensity on PYY, GLP-1 and hunger in healthy, active females.**

### Abstract

Despite a short duration of exercise, sprint interval training (SIT) can reduce body fat. This may be influenced by exercise-induced alterations in energy regulating (ER) pathways that reduce energy intake (EI). Little is known about how exercise intensity affects this pathway and ER hormones in women. This study compared the acute response of glucagon-like peptide-1 (GLP-1), peptide tyrosine-tyrosine (PYY), and self-reported hunger between a SIT cycling session and a moderate-intensity continuous training (END) session in females. **METHODS:** Nine healthy, active female volunteers completed three sessions in a randomized, crossover design. Participants completed one of the three sessions 1-h after a standardized breakfast: 1) cycling for 30-min at 65%  $VO_{2max}$  (END), 2) 6 x 30-second maximal effort cycling sprints with 4-min active recovery between SIT, or 3) resting control (CTRL). Sessions were completed one week apart in the early follicular phase, between days one and ten of the menstrual cycle. GLP-1 and PYY were measured via blood samples drawn pre-exercise, immediately post-exercise, and 90-min into recovery. Participants rated hunger on a visual analogue scale (VAS) at four time points during the session. Differences between sessions and across time were analyzed using repeated measures ANOVA. **RESULTS:** Circulating levels of GLP-1 were significantly higher in both SIT and END sessions ( $p < 0.05$ ) compared to CTRL, with no difference between SIT and END. There was a significant session by time interaction ( $p = 0.004$ ). PYY concentration did not differ between sessions ( $p = 0.249$ ) however, concentrations were higher at immediately post-exercise compared to 90-min

into recovery, in both exercise sessions and the control. Hunger was greater 90-min into recovery than immediately pre- ( $p=0.004$ ) and post- ( $p=0.002$ ) exercise, with no difference between exercise sessions. **CONCLUSIONS:** Our results suggest that satiety hormone GLP-1 is elevated post-exercise in women, however exercise intensity did not affect this response. Hunger ratings were not affected by exercise. The concentration of these hormones did not differ between traditional endurance exercise and high intensity sprint interval exercise.

The effect of exercise intensity on PYY, GLP-1 and hunger in healthy, active females.

## **Introduction**

In order to develop evidence-based guidelines for weight management, it is important to understand how exercise affects energy balance (EB). Energy intake (EI) regulation is a complex pathway that includes meal initiation, meal termination, meal frequency, nutrient intake and long-term regulation of EI in relation to body energy requirement (Valassi, Scacchi, & Cavagnini, 2008). Each of these components are experienced and acted upon as a result of the integration of various physiological and psychological factors, including hormonal signaling. The gastric peptide, ghrelin, stimulates appetite and is found in high concentration in a fasted state and low concentration when fed (Ariyasu et al., 2001; Callahan et al., 2004). Appetite suppressing hormones peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) are found in higher concentration postprandially and elicit satiety (Batterham et al., 2002; Elmquist, Maratos-Flier, Saper & Flier, 1998). A change in these hormones stimulates a cascade of events eventually terminating at neurotransmitter receptors in the hypothalamus, affecting appetite and subsequent EI.

Sprint interval training (SIT) is an exercise protocol that involves brief bursts of “all-out” cycling efforts, followed classically by 4-min of active rest (Gibala et al., 2006; Hazell et al., 2010). Classic endurance (END) exercise involves maintaining steady state intensity for an extended time, usually greater than 30-min. In this study, END exercise was performed at 65%  $VO_{2max}$ . SIT has been found to elicit adaptations that are similar to END with a smaller time commitment, including body fat loss (Burgomaster et al., 2006; Gibala et al., 2006; Helgerud et al., 2007; MacPherson, Hazell, Olver, Patterson &

Lemon, 2011). Little is known about the acute effect of these two forms of exercise on appetite regulating hormones and the resulting perception of hunger in women.

Conflicting results have been obtained in male subjects, with some studies showing that energy expenditure (EE) from exercise leads to an increased EI in the hours following, while others found short-term appetite suppression following exercise, dependent on exercise intensity (Durrant, Royston & Wloch, 1982; King, et al., 1994). Several studies have found increases in GLP-1 and PYY with END in males (Cooper et al., 2011, Deighton, Barry, Connon & Stensel, 2013, Kawano et al., 2013; Mackelvie et al., 2007; Martins, Morgan, Bloom & Robertson, 2007; O'Conner et al., 1995; Ueda et al., 2009a), with only a few examining the response of these hormones to SIT (Broom, Stensel, Bishop, Burns & Miyashita, 2007; Ueda et al., 2009a). The aforementioned studies utilized only male participants. In females, concentrations of PYY and GLP-1 have been shown to increase following high intensity END exercise (Hagobian et al., 2013; Larson-Meyer et al., 2012). To date, there have been no studies that have examined and compared this response following END exercise and SIT in females. While exercise is an effective method of increasing EE, and is often encouraged for weight management, its efficacy in appetite suppression and weight loss may vary with exercise intensity. Therefore, the aim of the present study was to compare the PYY, GLP-1, and hunger response between a traditional aerobic exercise session and a SIT exercise session in women.

## **Materials and Methods**

### **Participants**

Thirteen healthy, active females volunteered and provided written informed consent to participate. Four participants withdrew from the study for a variety of reasons, including: two because lack of time, one who moved cities and one where there were problems with obtaining two blood draws. This left nine participants available for analysis. All were healthy as assessed by the physical activity readiness questionnaire (PAR-Q), and were recreationally active such that they were capable of completing the physical demands of the study (CSEP, 2011). Recreationally active was classified as participating in moderate intensity exercise for a minimum of 30-min, three times per week. None of the participants were smokers or currently taking prescribed medication (aside from hormonal contraceptives including: combination pill, progesterone only pill, and inter-uterine device), and all were screened for history of diabetes, eating disorder, drug or alcohol abuse, coronary heart disease, food allergies, and medication use (specifically for those known to affect appetite, hypertension or induce weight loss). To control for phase of menstrual cycle, all participants, including those on oral contraceptives, completed all sessions during the early follicular phase (days 1-10) of the menstrual cycle, based on self-reported onset of menstruation.

### **Experimental Design**

Plasma concentrations of PYY and GLP-1 and subjective ratings of hunger were examined using a randomized crossover design. Subjects acted as their own control and completed three experimental sessions (CTRL, END, and SIT), which were performed in

a randomized order separated by at least one week. All sessions took place on the same day of the week.

### **Familiarization Session**

Prior to the experimental sessions, each participant attended a familiarization session in which they were screened for exclusion criteria and introduced to the study protocol and equipment. At this time anthropometric data (height, weight, and body composition) were collected and a graded maximum oxygen uptake test was performed.

**Anthropometry.** Participants had their height (nearest 0.1 cm) and body mass (nearest 0.1 kg) measured using a mechanical beam scale (Health-o-meter Professional, Sunbeam Products, Inc., Illinois, USA). Body mass index (BMI) was calculated as body mass (kg) over height (m) squared. Body density was calculated from skinfold measures obtained using a seven-site formula and the Siri equation to determine body fat percentage (Jackson, Pollock & Ward, 1980).

**Maximal oxygen uptake test.** Maximal oxygen uptake ( $VO_{2max}$ ) was measured directly using an online breath-by-breath gas collection system (Quark CPET, Cosmed, Chicago, Illinois, USA). The test followed a graded protocol to exhaustion on a mechanically braked cycle ergometer (model 874-E, Monark Exercise, Stockholm, Sweden). Following an incorporated 5-min warm up at 70 rpm and 1 kg resistance, participants maintained a 70 rpm cadence with 0.5 kg resistance added every 2-min until volitional fatigue was reached or 70 rpm could no longer be maintained (Taylor, Buskirk, & Henschel, 1955). Heart rate (HR) was measured throughout the test using a Polar HR monitor (FT7 - Polar Electro Oy, Kempele, Finland) and ratings of perceived exertion (RPE) were assessed simultaneously with each increase of resistance throughout the

duration of the test (Borg, 1973). At the end of the test,  $VO_{2max}$  (greatest 30-sec average) was established by the presence of a plateau in the  $VO_2$  or when two of the following criteria were obtained: (1) a respiratory exchange ratio (RER) value  $>1.15$ , (2) HR within  $\pm 10$  bpm of age predicted maximum HR ( $220 - \text{age}$ ), and/or (3) visible subject exhaustion (Midgley, McNaughton, Polman & Marchant, 2007). Upon determination of  $VO_{2max}$ , 65% of this value was calculated and used as the target exercise intensity during the END session. After the preliminary session participants were given at least one week to recover prior to their first experimental session.

### **Experimental Sessions**

Figure 2.1 shows an overview of the timeline of events for all experimental sessions. Participants were required to complete a 24-h dietary journal where they recorded their food intake, including the quantity of each food and beverage consumed for each meal, prior to their first experimental session. Participants were asked to replicate this same diet in the day prior to each subsequent session. On the morning of each session, participants arrived to the laboratory at 0800 h in a fasted state (no food or drink except water for a minimum of 10 h). Participants indicated their current level of hunger on a visual analogue scale (VAS) and then consumed a standardized breakfast equal to 4 kcal, 16.7 kJ per kg body weight. Breakfast consisted of an energy bar (250 kcal, 1046.0 kJ, 44 g carbohydrate, 9 g protein, and 5 g fat) and a rice cake (35 kcal, 146.4 kJ, 7 g carbohydrate, 1 g protein, and 0 g fat) with peanut butter (200 kcal, 836.8 kJ, 7 g carbohydrate, 8 g protein, and 15 g fat), with quantities determined as needed to achieve target caloric intake. Following breakfast, after 60 minutes of rest and prior to the start of the session, participants rested in a seated position while venous blood samples

were collected from the antecubital vein into precooled EDTA vacutainers (K2 EDTA, 10.8 mg – Franklin Lakes, NJ, USA). At this time, participants again indicated their current level of hunger using a VAS. Blood sample collection and hunger ratings were repeated again twice more during each session: immediately following exercise and 90-min into recovery from exercise.

Both exercise sessions were preceded by a 5-min standardized warm-up completed on the testing cycle ergometer (model 874-E, Monark Exercise, Stockholm, Sweden). Participants then completed the randomly selected session (a. CTRL, b. END 65%, or c. SIT) and upon completion rested for an additional 90-min prior to leaving the lab.

- a. SIT- Sprint interval cycling consisting of 6 sets of 30-second maximal effort bouts against a resistance equal to 10% of total body mass followed by 4-min of active recovery at a lower effort
- b. END- 30-min cycling at 65%  $\text{VO}_2$  max.
- c. Control (CTRL) – for the control session participants rested for the entire duration, but were permitted to read/write quietly.

## Measures

**Exercise Intensity.** RPE was assessed throughout END sessions every 5-min (Borg, 1973). RPE during SIT bouts was assumed to be maximal. HR was measured throughout exercise sessions using a polar HR monitor. One-min expired air samples were measured every 4-min during END bouts by computerized gas exchange analysis. The face mask was removed after one-min of collection and replaced after 4-min of

cycling had elapsed. Workload was adjusted accordingly if oxygen consumption varied from target intensity. Oxygen consumption was not measured during SIT.

**Hunger.** Hunger ratings were assessed using multiple VAS throughout each session. Participants were asked to indicate their current level of hunger with a dash along a 100mm line -the left side representing “not hungry” and the right representing “very hungry” (Parker et al., 2004). Participants had access to water *ad libitum* throughout the session.

**Hormones.** Blood samples were collected and sixty microliters of protease inhibitor cocktail (EDTA Free, 100x inDMSO – BioTool, Burlington, Ontario, Canada) was added to vacutainers. Samples were centrifuged at 1780 rpm for 10-min at 4°C. Plasma aliquots were stored at -80°C for later analysis.

### **Hormone Analysis**

The concentration of PYY in the test plasma samples was determined using Millipore human PYY total ELISA kits (EMD Millipore PYY Total ELISA Kit, Millipore Corporation, Billerica, MA, USA) and the concentration of GLP-1 was determined using Millipore GLP-1 total ELISA kits (EMD Millipore GLP-1 Total ELISA Kit, Millipore Corporation, Billerica, MA, USA) following the manufacturer’s instructions. The sensitivity of the assays was 1.4 pg/mL for PYY and 1.5 pmol/50 µL for GLP-1. All samples were assayed in duplicate and samples from one participant session were analyzed in the same assay to minimize the effects of inter-assay variation. Intra-assay variation was 5.8±1.9% and 4.5±2.3% for GLP-1 and PYY respectively, and inter-assay variation was 8.0±4.8% and 7.3±3.6% for GLP-1 and PYY, respectively.

## **Statistical Analysis**

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software v22.0 for Windows (SPSS, Chicago, IL). Hormone concentrations were normalized to baseline values and analyzed as absolute change in concentration from baseline. Differences in concentrations of GLP-1 and PYY across time and between sessions were examined using two-way repeated measures analysis of variance (ANOVA). For significant main effects, a Bonferroni adjustment was used for multiple pairwise comparisons. The Pearson product moment correlation coefficient was used to examine relationships between hormone concentrations and hunger ratings. Statistical significance was set at  $p < 0.05$ . All results are presented as mean  $\pm$  standard deviation, with the exception of figures where standard error is presented.

## **Results**

### **Subjects**

Nine females completed all experimental session, participant characteristics were: age:  $30.5 \pm 7.9$  y, height:  $1.75 \pm 0.15$  m, weight:  $72.4 \pm 2.0$  kg, BMI:  $23.5 \pm 2.8$  kg/m<sup>2</sup>, body fat%:  $22.8 \pm 4.3$ %, and  $VO_{2max}$ :  $40.7 \pm 5.4$  ml/kg/min.

### **Missing Data**

Data from blood samples are missing for one participant at the post-exercise time point for two of three sessions due to complications with obtaining blood via antecubital venipuncture (i.e. occasional clotting, venous spasm, or inadequate venous return). In these cases, hormone concentrations from the first two blood draws in the session were averaged and used.

### **Exercise Intensity**

During the END session, oxygen consumption reached steady state at ~65% of  $VO_{2max}$  ( $27.37 \pm 3.0$  ml/kg/min). The peak HR achieved was significantly higher during SIT ( $171.6 \pm 8.1$  bpm) versus END ( $153.1 \pm 72.6$  bpm,  $p=0.003$ ), however average HR did not differ significantly between exercise sessions (END=  $137.3 \pm 14.8$  bpm; SIT=  $135.5 \pm 13.9$  bpm,  $p=0.774$ ;

### **Plasma GLP-1 Response to Exercise**

Circulating concentrations of total GLP-1 during all three experimental sessions, presented as change from baseline, can be seen in Figure 2.2. On average, GLP-1 concentration increased  $42.0 \pm 17.2\%$  after exercise during END sessions and  $24.92 \pm 5.9\%$  in SIT. The repeated measures ANOVA found a significant main effect of session ( $p=0.004$ ). The concentrations of GLP-1 did not differ significantly between END exercise at 65% of  $VO_{2max}$  compared to SIT, however both END ( $p=0.007$ ) and SIT ( $p=0.015$ ) sessions resulted in significantly greater concentration change of GLP-1 compared to CTRL. Although there was no main effect for time ( $p=0.273$ ), there was a significant session by time interaction ( $p=0.004$ ) with GLP-1 increasing during both exercise sessions but decreasing steadily during the CTRL session. Absolute GLP-1 concentration is presented in Table 2.1.

### **Plasma PYY Response to Exercise**

When PYY was calculated as absolute change from baseline, there was a significant main effect of time ( $p=0.007$ ), with pairwise comparisons indicating lower circulating PYY concentrations at recovery compared to post-exercise ( $p=0.047$ ). There was no significant difference in PYY concentration between experimental sessions

( $p=0.249$ ) and no significant session by time interaction ( $p=0.256$ ), as displayed in Figure 2.3.

### **Hunger Ratings**

Figure 2.4 displays the average relative hunger in all three sessions on a scale of 1-100. There was a significant main effect of time ( $p<0.001$ ) with pairwise comparisons indicating higher hunger ratings before breakfast compared to immediately pre- and post-exercise. Additionally, subjective hunger ratings were greater at recovery than pre-exercise ( $p=0.004$ ) and post-exercise ( $p=0.002$ ). There was no difference in hunger between sessions ( $p=0.596$ ).

### **Correlations**

Correlations between hunger ratings and satiety hormones were weak and non-significant ( $p>0.05$ ).

## **Discussion**

To our knowledge, this is the first study to compare the GLP-1 and PYY response to endurance and sprint interval exercise in women. We found that GLP-1 increased in response to exercise compared to CTRL with no difference between END or SIT exercise. PYY concentrations were increased post-exercise compared to recovery with no difference between sessions. As expected, feeding influenced hunger ratings but again, there was no difference between exercise or control sessions.

Exercise has been shown to alter the concentration of appetite-regulating hormones PYY and GLP-1, as well as to have influence on appetite control (Martins et al., 2007; Ueda et al., 2009a). The majority of this research has focused on END exercise

in men. This study extends these findings by examining the effects of exercise on appetite-regulating hormones and hunger following both SIT and END in women. Past studies have demonstrated an increase in PYY and GLP-1 concentration following END exercise, though in men these results have been transient (Beaulieu, Olver, Abbott & Lemon, 2015; Hagobian et al., 2013; Larson-Meyer et al., 2013; Ueda Yoshikawa, Katsura, Usui & Fujimoto, 2009b). Elevated concentrations of these hormones are often accompanied by decreased perceptions of hunger (Broom, Stensel, Bishop, Burns & Miyashita, 2007; King et al., 2007, Ueda et al., 2009a). Here, we found no difference in PYY concentration between exercise and control sessions and no correlation between PYY concentration and hunger. Similar to previous research in females we observed an increase in GLP-1 concentration following END and SIT exercise (Larson-Meyer et al., 2012). The increase in GLP-1 that was observed was not associated with changes in hunger. The lack of association between hunger and PYY and GLP-1 illustrates that the suppression of the perception of hunger following exercise in previous research is not dependent only on concentrations of GLP-1 and PYY. These hormones are only one factor that can influence appetite. Other hormones and factors, such as exercise mode and duration, also contribute to post-exercise hunger and should be considered. It is also possible that our measure of hunger was not sensitive enough (Parker et al., 2004).

SIT has been shown to induce adaptations in both aerobic and muscular performance, similar to that of END training, with a lesser time commitment (Burgomaster et al., 2008; Gibala et al., 2006). END training is known to elicit reductions in body fat mass, however, research has demonstrated that SIT may result in comparable, if not greater, effects on body fat (Heydari, Freund & Boutcher, 2012; MacPherson et al., 2011; Shaw, Gennett, O'Rourke & DelMar, 2006). Interestingly, MacPherson et al.

(2011) found that fat mass decreased significantly in women following six weeks of END training (average weight-loss of 1.4 kg), but not following a SIT program (average weight-gain of 0.3 kg). Conversely, fat loss was observed in women participating in a SIT protocol, but still not close to the 3.6 kg that males lost while following a similar procedure (Hazell et al., 2014; MacPherson et al., 2011). While men and women experience similar relative EE from SIT it has been suggested that increased appetite and compensatory EI post-exercise may occur in women, diminishing the efficacy of SIT for fat loss (Hagobian et al., 2013; Townsend, Couture & Hazell, 2014). With the increasing popularity of SIT, we felt it was important to investigate the response of appetite regulating hormones to this mode of exercise compared to traditional END exercise in women. Here we see that exercise caused an increase in satiety hormone GLP-1, at least transiently, regardless of exercise intensity. Furthermore, PYY concentrations were elevated with SIT, although not statistically significant, which may be due in part to small sample size. Since exercise-intensity did not significantly affect the concentrations of GLP-1 and PYY, it appears that these hormones are not the reason that some studies have shown that females do not respond to SIT the same as END for fat loss (MacPherson et al., 2011).

A strength of this project is that we have extended previous research to a female population. This provides insight into how the hormonal profile of appetite-regulating peptides and hunger may vary between exercise intensities that are often prescribed for weight loss. The present study is limited mainly due to small sample size. Additionally, the sample was comprised solely of young, healthy individuals of reproductive age who were currently moderately active. Therefore, the findings of this study may not generalize to inactive populations, elderly, obese, or women in menopause. Additionally, the SIT

exercise protocol was very intense and may not be suitable for all populations. Consequently, the practicalities of recommending this type of exercise to the general population should be considered, although it has been shown to be tolerated well by some older untrained individuals (Willoughby et al., 2015). Furthermore, while we instructed participants to consume the same EI in the 24 hours prior to sessions, and to refrain from strenuous exercise and alcohol in the hours leading up to the session, their compliance is unknown. Lastly, we measured total GLP-1 and PYY and not the active forms GLP-1<sub>7-36</sub> and PYY<sub>3-36</sub>. GLP-1 exists primarily in two forms, the majority of the circulating biologically active form being GLP-1<sub>7-36</sub>. This form is rapidly metabolized to GLP-1<sub>9-36</sub> following its secretion, which is the predominant form of GLP-1 post-prandially (Vahl, Paty, Fuller, Prigeon & D'Alessio, 2003). It is possible that analysis of this form of GLP-1 would result in different findings. Measuring the response of appetite-stimulating hormone ghrelin would also provide additional insight into exercise-induced hunger changes. Additional work should be considered measuring active forms of each hormone while investigating the effect of practicable exercise protocols on EB for obese and overweight populations. This is of particular importance as these are the populations for which weight-management approaches are the most clinically relevant.

In conclusion, this is the first study to compare the response of GLP-1, PYY, and hunger to endurance exercise and sprint interval exercise in women. The primary finding of this study is that GLP-1 increased after both END and SIT exercise, which may have the potential to influence satiety. Future studies should be conducted to further examine the potential influence of exercise-induced changes in PYY and GLP-1 on hunger and EI. Additionally, future studies should observe whether the acute response of these hormones

and associated hunger following exercise translates into fat loss over time, with potential implication on weight-management and health prescription.

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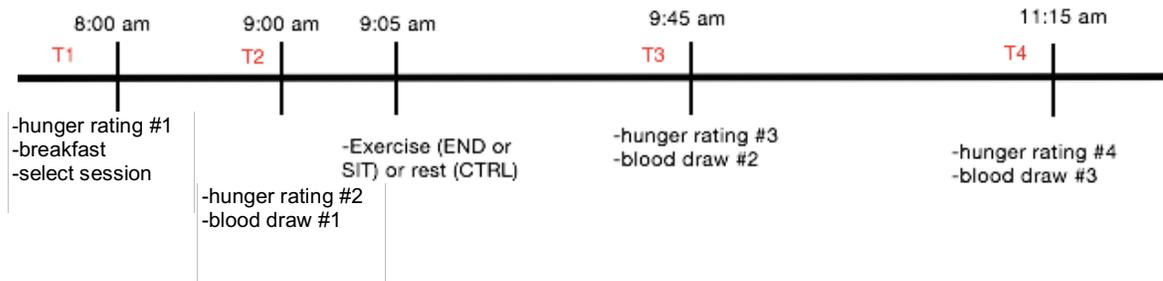
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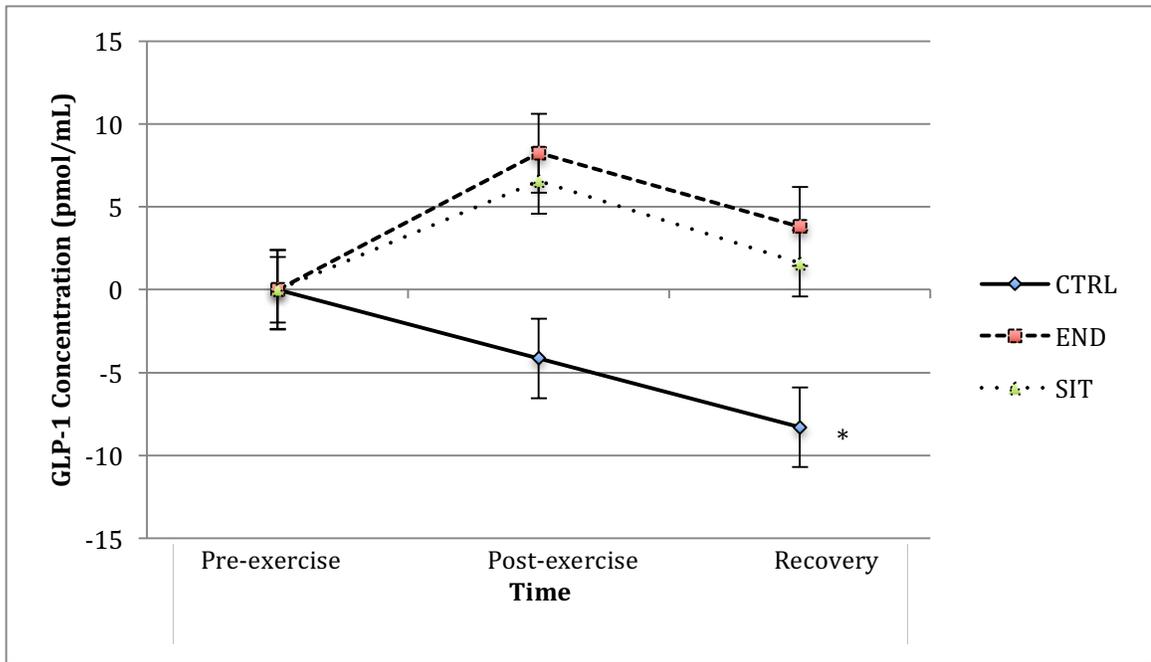


*Figure 2.1.* Timeline of events during experimental sessions. VAS: visual analogue scale; CTRL= control session; END = 30-min continuous endurance exercise at 65%  $VO_{2max}$ , SIT= sprint-interval training consisting of 6x 30-sec maximal effort cycling sprints separated by 4-min recovery; T1= Breakfast; T2=Pre-exercise; T3=Post-exercise; T4=Recovery

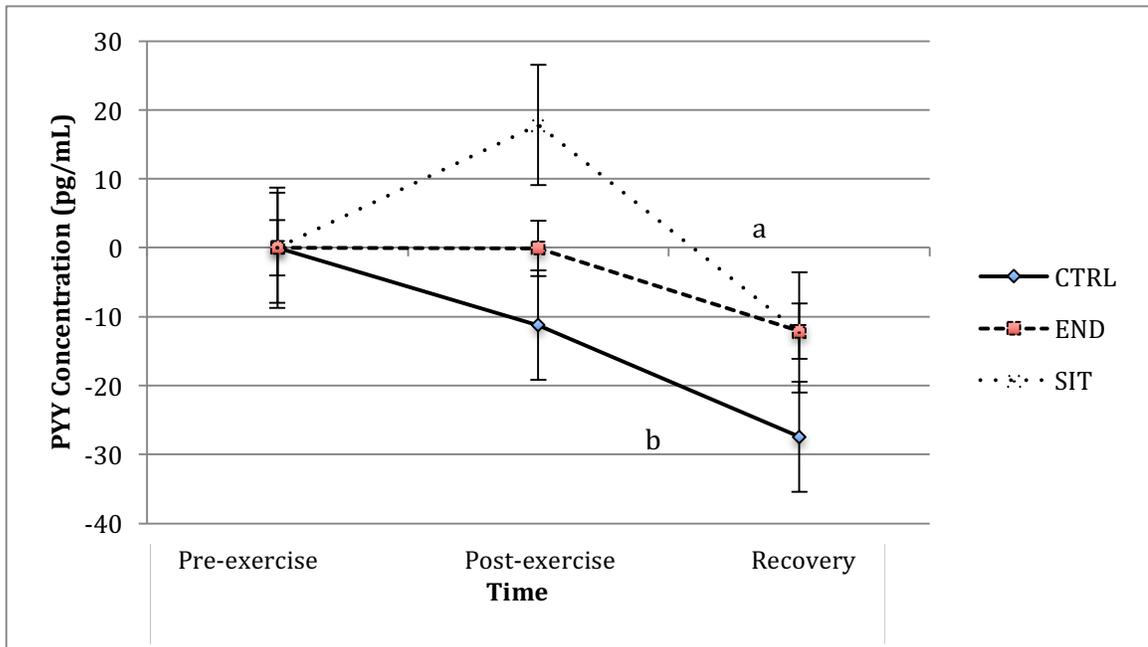
**Table 2.1: Concentration of GLP-1 and PYY concentration during sprint intervals, endurance cycling and rest (mean  $\pm$  S.D.) in women.**

Session	GLP-1 (pmol/mL)			PYY(pg/mL)		
	Pre-exercise	Post-exercise	Recovery	Pre-exercise	Post-exercise	Recovery*
CTRL	30.9 $\pm$ 13.6	26.8 $\pm$ 15.4	21 $\pm$ 11.9	123.9 $\pm$ 38.3	112.6 $\pm$ 32.3	96.4 $\pm$ 31.2
END	22.2 $\pm$ 12.8	30.4 $\pm$ 15.7	26 $\pm$ 14.7	107 $\pm$ 59.6	106.9 $\pm$ 59.6	93.9 $\pm$ 60.6
SIT	27.6 $\pm$ 9.3	34.2 $\pm$ 12.1	29.2 $\pm$ 10.9	102 $\pm$ 52.3	119.8 $\pm$ 52.7	89.7 $\pm$ 42.4

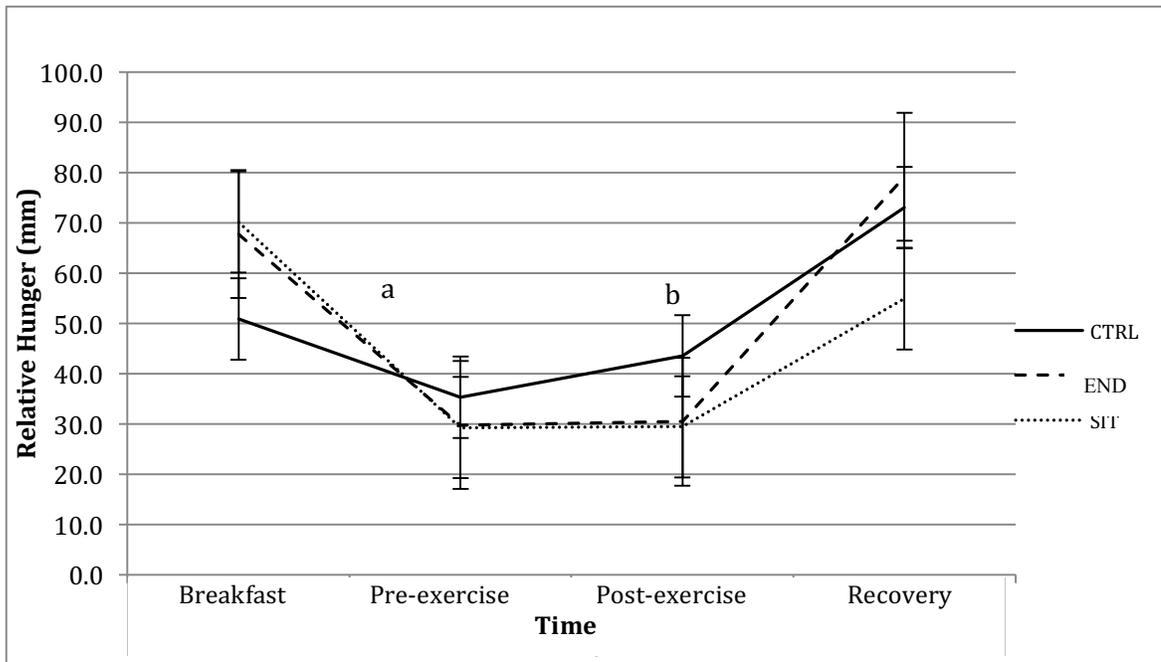
CTRL: resting control, END: continuous endurance cycling at 65%  $VO_{2max}$ , SIT: sprint-interval training; 6x30-sec sprints with 4-min active recovery; \*main effect of time: post-exercise greater than recovery ( $p < 0.05$ )



*Figure 2.2.* Change in GLP-1 concentration (mean  $\pm$  S.E.) in all experimental sessions (CTRL= control, END= endurance exercise at 65%  $VO_{2max}$ ; SIT= sprint-interval training) across time. Values are presented as change from baseline. \*: main effect for session: CTRL lower than END and SIT ( $p < 0.05$ ).



*Figure 2.3.* Absolute change in PYY concentration (mean  $\pm$  S.E.) in all experimental sessions (CTRL= control, END=endurance exercise at 65%  $VO_{2max}$ ; SIT= sprint-interval training) across time. Values are presented as change from baseline. a: Change in PYY concentration from pre-exercise to recovery is less than pre-exercise to post-exercise in SIT ( $p<0.05$ ); b: Change in PYY concentration from pre-exercise to recovery is greater than pre-exercise to post-exercise in CTRL ( $p<0.05$ ).



*Figure 2.4.* Subjective hunger ratings (mean  $\pm$  S.E.) throughout control (CTRL), endurance cycling at 65%  $VO_{2max}$  (END) and sprint-interval training (SIT). Values are represented as hunger change from baseline. a: Pre-exercise < Breakfast and Recovery ( $p < 0.05$ ); b: Post-exercise < Breakfast and recovery ( $p < 0.05$ ).

### **Chapter 3: Sex differences in the response of PYY and GLP-1 appetite regulating hormones to moderate intensity aerobic cycling.**

#### **Abstract**

Aerobic endurance exercise at moderate intensity is often prescribed for health, weight-loss and prevention of metabolic disease. Weight-loss may result, in part, from alterations in the energy-regulating (ER) hormones responsible for appetite regulation and the response to this type of exercise may differ between males and females. The present study investigates the acute response of satiety hormones glucagon-like peptide-1 (GLP-1) and peptide tyrosine-tyrosine (PYY), and self-reported hunger in males and females following 30-min of moderate-intensity continuous endurance (END) cycling at 65%  $VO_{2max}$ . **METHODS:** Nineteen active adults (n=9 females, n=10 males) volunteered and completed two sessions in a randomized, crossover design. Participants completed one of the two sessions 1-h after a standardized breakfast: cycling for 30-min at 65%  $VO_{2max}$  (END) or resting control (CTRL). Sessions were separated by one week and were completed in the early follicular phase, between days 1 and 10 of the menstrual cycle, for women. GLP-1 and PYY were measured via blood samples at three times: pre-exercise, immediately post-exercise, and 90-min into recovery. Participants also rated their hunger at four times through the session using a visual analogue scale (VAS). Differences between sexes, sessions and across time were analyzed using mixed- model repeated measures ANOVA. **RESULTS:** END sessions elicited greater change in both GLP-1 (p=0.001) and PYY (p=0.017) concentration compared to CTRL. There was a session X sex interaction for GLP-1 change from baseline (p=0.007), but no difference in PYY between men and women (p=0.598). There was no effect of sex or session on hunger

ratings. Our results suggest that satiety hormones GLP-1 and PYY are changed with exercise, however this response does not appear to differ between males and females.

Sex differences in the response of PYY and GLP-1 appetite regulating hormones to moderate intensity aerobic cycling.

### **Introduction**

Many Canadian adults fail to meet the minimum physical activity (PA) guidelines recommended by the Public Health Agency of Canada and the Canadian Society of Exercise Physiology (CSEP). The Canadian Physical Activity Guidelines recommends that adults, age 18-64 years, partake in 150-min per week of moderate-to-vigorous intensity aerobic activity (CSEP, 2011). Endurance (END) protocols are typically the exercise prescription reflected in public health guidelines; such as 30-min moderate-intensity walking at least 5 days per week.

The effectiveness of exercise for weight loss in the absence of dietary restriction is a controversial topic. Though a great deal of individual inter-variability exists, some individuals find weight loss from exercise alone difficult to achieve (Donnelly et al. 2003; Donnelly & Smith, 2005; Hall et al. 2012; King et al. 2007). Explaining this difficulty requires an improved understanding of how exercise affects energy intake (EI). It is possible that individuals who are unsuccessful with weight loss may have increased perceptions of hunger after exercise that encourages them to increase EI, often negating the energy deficit created by exercise (King, Hopkins, Caudwell, Stubbs, & Blundell, 2008).

Women have been shown to respond differently than men to exercise in terms of fat loss from exercise (Donnelly et al., 2003; Oscai, Mole & Holloszy, 1971). Physiological mechanisms that maintain body fat are more effective in women than men (Hagobian et al., 2009). This has been illustrated in various studies that have found reductions in body fat in males following structured exercise programs at moderate and

high intensity, with no change in body fat in females following identical protocols (Despres, Allard, Tremblay, Talbot & Bouchard, 1984; Hill, Sparling, Shields & Heller, 1987; MacPherson, Hazell, Olver, Paterson & Lemon, 2011; Potteiger, Jacobsen, Donnelley & Hill, 2003). These data are supported by similar research that reported sex differences in body fat loss after high-intensity exercise training (Henderson et al., 2008; MacPherson et al., 2011). Differences in exercise-induced fat loss between men and women may be due to compensatory changes in appetite following exercise.

Concentrations of appetite suppressing hormones peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) have been measured following END exercise. Levels of both PYY and GLP-1 have been found to increase following END exercise (Cooper et al., 2011; Deighton, Barry, Connon & Stensel, 2013; Kawano et al., 2013; Mackelvie et al., 2007; Martins, Morgan, Bloom & Robertson, 2007; O'Conner et al., 1995; Ueda, Yoshikawa, Katsura, Usui & Fujimoto, 2009). It is important to note that the aforementioned studies, with the exception of Hagobian et al. (2013), utilized only male participants. One study found END exercise at 60%  $VO_{2max}$  increased PYY concentrations in females but had no effect in males, although this difference was not significant (Hagobian et al., 2013). They concluded no effect of sex on PYY concentrations. More research is needed to determine if exercise causes a differing response in appetite-regulating hormones between men and women. Therefore, the purpose of this study was to investigate the sex difference in perceived hunger, PYY concentration and GLP-1 concentration in response to endurance exercise. It was expected that GLP-1 and PYY concentration would be elevated with exercise in men and women, in men more-so than women.

## **Materials and Methods**

### **Participants**

Nineteen, active adults (n=9 females, n=10 males) volunteered to participate in this study. All participants were healthy as assessed by the physical activity readiness questionnaire (PAR-Q), and provided written, informed consent (CSEP, 2011b).

Participants were physically active, such that they could meet the physical demands of the study. Recreational activity was defined as participating in moderate intensity physical activity for a minimum of 30-min, three times per week. Participants were non-smokers, not currently taking prescription medication known to affect appetite, hypertension, or induce weight loss, and were screened for: history of disordered eating, drug or alcohol abuse, food allergies, and presence/risk of chronic disease (i.e. diabetes, coronary heart disease, etc.). To control for phase of menstrual cycle, female participants completed all sessions during days 1 and 10 of their menstrual cycle, based on self-reported onset of menstruation.

### **Experimental Design**

The effects of exercise on plasma concentrations of PYY and GLP-1 and measures of subjective hunger in men and women were analyzed using a randomized crossover design. Participants completed two sessions (1) control and 2) moderate intensity continuous exercise) that were performed in a random order, at least one week apart.

### **Familiarization Session**

Participants attended a familiarization session at least one-week prior to the start of experimental sessions. At this time, they were screened for exclusion criteria and were

introduced to the study protocol and equipment. Anthropometric data (height, weight, and body composition) were collected and a graded exercise test was performed to obtain maximum oxygen consumption ( $VO_{2max}$ ).

**Anthropometry.** Height (nearest 0.1cm) and body mass (nearest 0.1kg) were measured using a mechanical beam scale (Health-o-meter Professional, Sunbeam Products Inc., Illinois, USA). Body mass index (BMI) was calculated as body mass (kg) over height (m) squared. Jackson & Pollock (1978) seven-site skin formula and the Siri equation were used to estimate relative body fat from skinfold measures (Jackson, Pollock & Ward, 1980).

**Maximal Oxygen Uptake Test.** Participants completed a graded protocol to exhaustion on a mechanically braked cycle ergometer (model 874-E, Monark Exercise, Stockholm, Sweden) to determine  $VO_{2max}$ .  $VO_{2max}$  was measured directly using an online breath-by-breath gas collection system (Quark CPET, Cosmed, Chicago, Illinois, USA). Prior to the start of the test, a silicon facemask was placed over the nose and mouth, harnessed around the head, and once checked for leaks, was connected to the gas collection system mentioned above. Following an incorporated 5-min warm-up at 70 rpm and 1 kg resistance, participants maintained a 70 rpm cadence with 0.5 kg added resistance every 2-min until volitional fatigue was reached or a 70 rpm cadence could no longer be maintained, as suggested by Taylor, Buskirk and Henschel (1955). Heart rate (HR) was measured throughout the test using a Polar monitor (FT7-Polar Electro Oy, Kempele, Finland). Ratings of perceived exertion (RPE) were assessed at the time of each increase of resistance for the duration of the test (Borg, 1973).  $VO_{2max}$  (greatest 30-second average) was established at the end of the test by the presence of a plateau in the  $VO_2$  or when two of the following criteria were obtained: (1) a respiratory exchange ratio

(RER) value  $>1.15$ , (2) HR within  $\pm 10$  bpm of age predicted maximum HR ( $220 - \text{age}$ ), and/or (3) visible subject exhaustion (Midgley, McNaughton, Polman & Marchant, 2007). Participants were given at least one-week of recovery following the preliminary session, before starting the exercise sessions.

### **Experimental Sessions**

Figure 3.1 shows the timeline of events for each session. In the 24 hours prior to the first session, participants were asked to complete a dietary food and beverage intake journal and replicate this same diet in the day prior to their second trial. Participants arrived to the laboratory in a fasted state (no food or drink except water for a minimum of 10-h) at 0800-h. After indicating their current level of hunger, participants consumed a standardized breakfast equal to 4 kcal, 16.7 kJ, per kg of body mass. Breakfast consisted of an energy bar (250 kcal, 1046 kJ, 44g CHO, 9 g PRO, 5 g fat) plus a rice cake (35 kcal 146.4 kJ, 7 g CHO, 1 g PRO, 0 g fat) and peanut butter (200 kcal, 836.8 kJ, 7 g CHO, 8 g PRO, 15 g fat) with quantities determined as needed to achieve the target caloric intake. Participants rested quietly for 60-min followed by the first blood sample and again indicated their hunger along a second visual analogue scale (VAS). Participants then completed the randomly selected session (a. CTRL or b. END @ 65%  $\text{VO}_{2\text{max}}$ ) and upon completion, rested for an additional 90-min prior to leaving the lab. The exercise session was preceded by a 5-min standardized warm-up completed on the testing cycle ergometer. Two additional blood samples and hunger ratings were collected throughout the sessions.

## Measures

### Exercise Intensity

RPE was assessed throughout the END sessions every 5-min. RPE during SIT bouts was assumed to be maximal. HR was measured throughout exercise using a Polar HR monitor. Oxygen uptake was monitored via one-minute expired gas samples, measured every 4-min by computerized gas exchange analysis and cycling workload was adjusted as needed to maintain target intensity.

**Hunger.** Hunger ratings were assessed using a VAS. Participants were asked to indicate their current level of hunger with a vertical line along a 100mm scale. The ends of the scale represented extremes, with the left representing “not hungry” and the right representing “very hungry” (Parker et al., 2004). Participants had access to water *ad libitum* throughout the session.

**Hormones.** Blood samples were collected and sixty microliters of protease inhibitor cocktail (EDTA Free, 100x inDMSO – BioTool, Burlington, Ontario, Canada) was added to 6mL whole blood. Samples were centrifuged at 1780 rpm for 10-min at 4 degrees Celsius. Plasma aliquots were stored at -80 degrees Celsius for later analysis.

### Hormone Analysis

Concentrations of total PYY and total GLP-1 were determined using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer’s instructions. The concentration of PYY in the test plasma samples were determined using Millipore human PYY total ELISA kits (EMD Millipore PYY Total ELISA Kit, Millipore Corporation, Billerica, MA, USA) and the concentration of GLP-1 was determined using Millipore GLP-1 total ELISA kits (EMP Millipore GLP-1 Total ELISA Kit, Millipore

Corporation, Billerica, MA, USA) following the manufacturer's instructions. All samples were assayed in duplicate and samples from each participant's individual sessions in one assay to minimize the effects of inter-assay variation. Intra-assay variation was  $4.9 \pm 1.1\%$  and  $4.7 \pm 2.8\%$  for GLP-1 and PYY respectively, and inter-assay variation was  $7.4 \pm 4.2\%$  and  $8.3 \pm 2.9\%$  for GLP-1 and PYY, respectively.

### **Statistical Analysis**

Data were analyzed using the Statistical Package for Social Science (SPSS) software ver. 22.0 for Windows (SPSS, Chicago, IL). A mixed-model three-factor (sex X session X time) ANOVA with repeated measures was used to examine differences in GLP-1, PYY and hunger between sexes, sessions and across time. If sex differences did not exist in hormone concentrations and hunger ratings, a two-factor ANOVA was used for men and women independently. A Pearson product moment correlation coefficient was used to determine associations between variables. By convention, significant differences were defined as  $p < 0.05$ , and a Bonferroni post hoc analysis was used when significance occurred. All results are presented as mean plus or minus standard deviation, with the exception of figures where standard error is presented.

## **Results**

### **Subjects**

Ten males and nine females were recruited and completed the preliminary  $VO_{2max}$  testing. All participants completed both experimental sessions. Their physical characteristics are presented in Table 3.1.

### **Exercise Intensity**

Oxygen consumption reached steady state during END exercise. There was no significant difference between the average exercise HRs between men ( $138.5 \pm 6.4$  bpm) or women ( $137.3 \pm 7.8$  bpm) ( $p=0.173$ ).

### **Plasma GLP-1 Response to Exercise**

There was no sex difference in absolute GLP-1 concentration ( $p=0.792$ ) or GLP-1 when calculated as change from baseline ( $p=0.359$ ). Absolute GLP-1 concentration is presented in Table 3.2. Figure 3.2 presents GLP-1 concentration change from baseline. Repeated measures ANOVA revealed a significant main effect of session ( $p=0.001$ ) and time ( $p=0.003$ ) for change in GLP-1 concentration. Pairwise comparisons indicated a greater change in GLP-1 with END compared to control (CTRL) and post-exercise compared to recovery. There was a significant session X sex ( $p=0.007$ ) and session X time X sex interaction ( $p=0.006$ ), which appeared to be due to GLP-1 change in the CTRL session among women (Figure 3.2). To examine this further, men and women were analyzed separately using 2 x 3 ANOVAs (session X time).

When analyzed separately, GLP-1 appears to respond to exercise differently in men and women. There was no effect of session on change in GLP-1 in men ( $p=0.467$ ). In women, however, GLP-1 increased by  $4.0 \pm 2.1$  pmol/mL from baseline to post-exercise during END and decreased by  $4.3 \pm 1.8$  pmol/mL from baseline post-exercise during CTRL. The ANOVA revealed a significant effect of session for women when GLP-1 was calculated as change from baseline ( $p=0.002$ ), as well as a significant session X time interaction ( $p=0.001$ ). Absolute GLP-1 concentration was not affected by session in women ( $p=0.960$ ). Additionally, there was no significant effect of time on GLP-1

concentration change in women ( $p=0.318$ ). Among men, GLP-1 increased post-exercise by  $5.5\pm 1.7$  pmol/mL, falling by  $5.9\pm 0.9$  pmol/mL to levels below baseline at recovery. When GLP-1 was analyzed as change from baseline in men, ANOVA revealed a significant main effect of time ( $p=0.001$ ), with no difference between CTRL and END sessions. Pairwise comparisons indicated that GLP-1 concentration change was greater at post-exercise compared to pre-exercise ( $p=0.034$ ) and recovery ( $p=0.001$ ).

### **Plasma PYY Response to Exercise**

There was no sex difference in absolute PYY concentration ( $p=0.858$ ) or in change in PYY concentration when normalized from baseline ( $p=0.598$ ). There was a main effect of session ( $p=0.017$ ) and time ( $p<0.001$ ) for PYY when calculated as change from baseline, with a greater change in PYY concentration in END compared to CTRL, and at pre-exercise compared to recovery ( $p<0.001$ ) and post-exercise compared to recovery ( $p<0.001$ ) (Figure 3.3).

When men and women were analyzed separately, using separate 2 x 3 ANOVAs (session X time), the PYY response to exercise appeared to differ. In men, PYY concentration was not different between exercise and control ( $p=0.122$ ). The PYY response in men was also not different between sessions when PYY was calculated as a change from baseline ( $p=0.205$ ). However, in women, PYY concentration decreased by  $13.9\pm 4.6$  pg/mL in CTRL and increased by  $4.1\pm 3.8$  pg/mL in END. ANOVA revealed that there was a significant main effect of session with pairwise comparisons indicating a greater decrease in PYY concentration from baseline in CTRL ( $p=0.040$ ). Absolute PYY concentration is shown in table 3.3. There was a main effect of time for PYY concentration normalized as change from baseline in both men and women, with PYY

concentration post-exercise greater than recovery ( $p < 0.05$ ) (Figure 3.3). There was no session X time interaction in men ( $p = 0.064$ ) or women ( $p = 0.104$ ).

### **Hunger Ratings**

Hunger ratings are reported on a scale of 1-100. Baseline hunger ratings following the 10-h fast were not different between sexes, as shown in Figure 3.4 (Male:  $38.6 \pm 4.9$ , Female:  $47.3 \pm 5.1$ ,  $p = 0.239$ ).

Examination of the perceptions of hunger over the course of both END and CTRL sessions revealed a significant effect of time on the hunger response for both males and females together (Figure 3.4; Breakfast:  $55.8 \pm 4.4$ , pre-exercise:  $27.2 \pm 4.3$ , post-exercise:  $27.9 \pm 3.9$ , recovery:  $60.8 \pm 4.6$ ), where hunger was suppressed immediately following the standardized meal and continually increased until 90-min post-exercise ( $p > 0.001$ ). There was no significant effect of session on perceptions of hunger (CTRL:  $39.8 \pm 4.5$ , END:  $46.2 \pm 4.4$ ,  $p = 0.415$ ).

### **Correlation between Hunger and Hormones**

Correlations between perceptions of hunger and satiety hormones were weak and non-significant in both men and women ( $p > 0.05$ ).

## **Discussion**

Exercise can influence appetite and concentrations of appetite-regulating hormones, including satiety hormones GLP-1 and PYY, though this may differ in men and women (Hagobian et al., 2009; Martins et al., 2007; Ueda et al., 2009). Most available research focuses on the effect of exercise on these hormones and appetite in male populations. This study extends these findings by comparing appetite-regulating

hormones and hunger following 30-min of cycling at 65%  $VO_{2max}$  in males and females. The primary finding of this study is that END exercise increases GLP-1 concentration when compared to control, though this difference was only really evident in women. Furthermore, the change in PYY concentration was greater during END cycling compared to CTRL, with no difference between men and women.

Acute bouts of exercise have been shown to elicit changes in the circulating concentration of PYY (Martins et al., 2007; Ueda et al., 2009). In agreement with previous research in males we saw an increased PYY concentration during END compared to CTRL (Deighton et al., 2013; Martins et al., 2007; Ueda et al., 2009). To the author's knowledge, there is no current research comparing the response of PYY to END in males and females. We did not observe a sex difference in PYY response to acute END exercise. Additionally, we observed an effect of time on concentration change in both PYY and GLP-1; which may have been influenced by feeding due to PYY's time to peak and plateau post-prandially.

In agreement with past research in both male and females, we observed an increase in GLP-1 concentrations following an acute bout of END exercise (Larson-Meyer et al., 2012; Mackelvie et al., 2007; O'Conner et al., 1995; Ueda et al., 2009). The present study demonstrated that GLP-1 levels are elevated with exercise in women, however not significantly different between CTRL and END sessions in men. In women, the concentration decreased continually over time in CTRL, but during cycling peaked immediately following exercise, then falling to levels still elevated compared to baseline after recovery from exercise.

In general, the initiation of aerobic exercise training has been shown to result in greater fat loss in men than in women (Donnelly et al., 2003; Oscai et al., 1971). When

participating in an aerobic exercise program at moderate and high-intensities, exercise has been associated with more fat loss in men than women (Despres et al., 1984; Donnelly et al., 2003; Hill et al., 1987; Potteiger et al., 2003). These findings are supported by similar research that revealed sex differences in body fat loss during high-intensity exercise (Henderson et al., 2008; MacPherson et al., 2011). One suggested reason for fat loss with exercise, in addition to EE, is due to elevated levels of satiety hormones, decreased appetite and decreased EI post-exercise, as seen in some available studies using male participants (Deighton et al., 2013; Howe, Hand & Manore, 2014; King, Wasse, Broom & Stensel, 2010; Ueda et al., 2009). This reduced EI coupled with increased exercise EE would further increase the energy deficit achieved through exercise, and with chronic exercise training, could result in increased fat loss. In contrast to previous research, which utilized only male participants, we did not observe reduced perceptions of hunger coinciding with elevated GLP-1 and PYY concentrations (Broom et al., 2009; Kawano et al., 2013). Additionally, we did not observe significant sex differences in the response of GLP-1 and PYY to END exercise that would prompt us to believe that men and women would experience exercise-induced satiety differently. However, we measured total forms of both hormones and active forms, which may have a greater influence on appetite than the total forms (Batterham et al., 2002; Mackelvie et al., 2007), may elicit different reactions. The previously researched sex differences in exercise-induced fat loss may be a result of mechanisms other than elevated PYY and GLP-1 affecting hunger and EI following exercise, such as meal consumption as reward for completion of exercise, a general misunderstanding of the energy demand of exercise and the energy provided by meals, or by a sex-difference in total energy expenditure during exercise.

Sex differences in exercise-induced fat loss may be facilitated by alterations in the endocrine response, including forms of satiety hormones GLP-1 and PYY, with potential implications on appetite and EI post-exercise. It has been suggested that women are more likely to match EI in response to exercise-induced EE in order to support the delicate balance between EB and reproductive capability (Howe et al., 2014). If EI chronically does not match the demand of energy requirements, women are at an increased risk for various health issues, including: stress fractures, suppressed immune response, and female athlete triad (amenorrhea, eating disorders, osteoporosis) (Howe et al., 2014). In addition, chronic energy deficit produces a cascade of hormonal consequences, including: inhibited gonadotropin-releasing hormone secretion, reduced pulsatile release of luteinizing hormone, suppressed ovulatory cycles and decreased libido (Wade & Jones, 2004). There appears to be no impact of energy deficit on reproductive success in men. In order to protect against the deleterious effects of sustained negative EB it has been suggested that exercise may lead to stimulated appetite and EI, as well as reduced EE in women compared to men (Hagobian et al., 2009).

To protect against energy deficit, we would expect to see alterations in appetite-regulating hormones in women that would maintain EB following increased EE with exercise, such as: elevated acylated ghrelin, lower insulin, leptin, PYY and GLP-1. This would defend body fat stores and preserve reproductive function in women. Our findings indicated no sex difference in plasma PYY and GLP-1 concentrations or hunger response to exercise, which contradicts the expected result built on the theory that women defend body fat for protection of reproductive health. Higher levels of GLP-1 are expected to increase feelings of satiety and therefore reduce EI, which, over the long-term results in body weight reduction. This may mean that the previously observed sex differences in fat

loss with exercise occurs as a result of mechanisms other than the response of satiety hormones GLP-1 and PYY to exercise EE, such as alterations in appetite stimulating hormone ghrelin, a misunderstanding calorie intake and calorie output, or hedonistic and behavioral eating habits.

Lastly, there was no significant association found between GLP-1 or PYY concentration and perceptions of hunger. This lack of association may reflect the modest role of endogenous GLP-1 and PYY in the overall regulation of hunger. It may also be that other factors, such as appetite regulating neuropeptide concentration or psychological drive for energy intake are more affected by exercise. Finally, our measures of hunger may not have been sensitive enough to detect a relationship.

While we practiced experimental control over participants 24-h EI in the day prior to sessions, their compliance is unknown and inconsistencies in food consumption could influence the circulation of PYY and GLP-1 in the day following. Additionally, we saw a large degree of variability in results, which may reduce our statistical power with a small sample size. Further work should be performed on a larger population to investigate the influence of protocols involving other types of exercise (e.g. resistance training, interval training) on these, and other, appetite-regulating hormones. In conclusion, change in plasma concentrations of PYY and GLP-1 were greater following exercise compared to control, with no significant differences between men and women. Additionally, there was no difference in perceived hunger in men and women following exercise. It is likely that mechanisms other than PYY and GLP-1 concentration are responsible for sex differences in exercise-induced fat loss.

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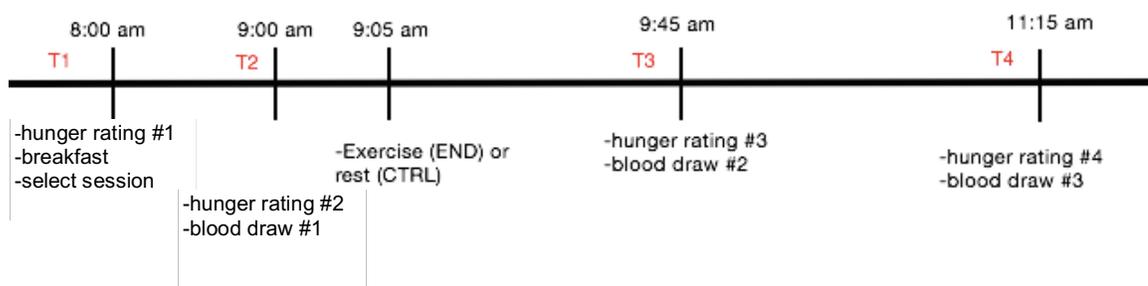
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**Table 3.1: Participant Characteristics**

	Female (n=9)		Male (n=10)	
	Mean	S.D.	Mean	S.D.
Age (years)	30.5	7.9	28.6	5.9
Height (m)	1.75	0.15	1.8	4.2
Body Mass (kg)	72.4	2	77.5	7.2
BMI (kg/m <sup>2</sup> )	23.5	2.8	23.9	2.2
Body Fat %	22.8	4.3	11.7	3.9
VO <sub>2max</sub>	40.7	5.4	46.8	4.8

*Note:* BMI: Body mass index, VO<sub>2max</sub>: Maximal oxygen uptake

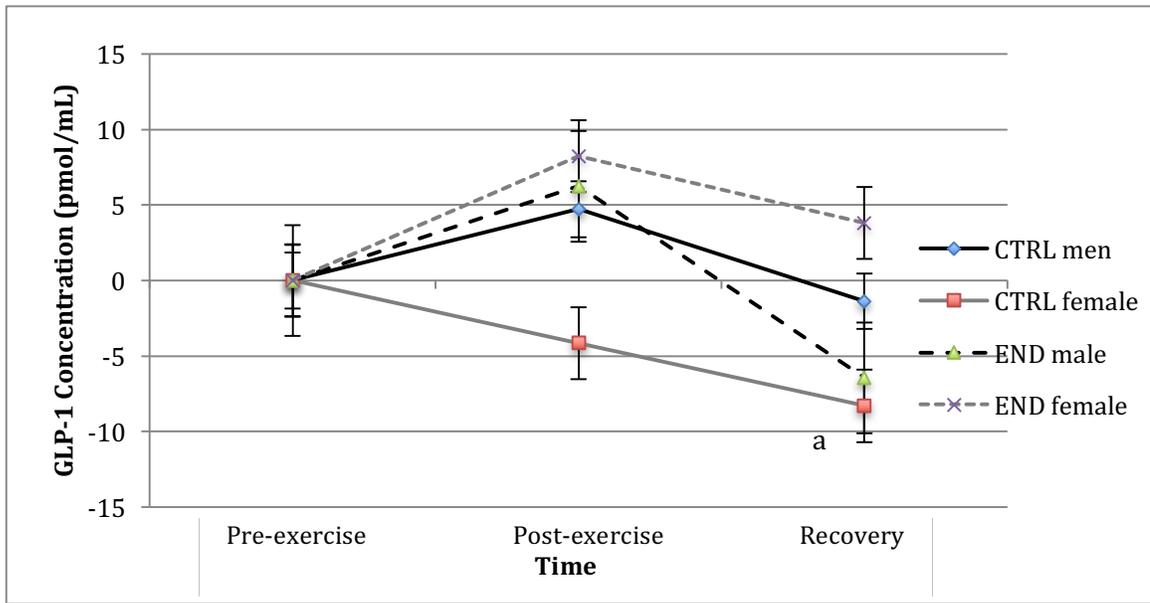


*Figure 3.1.* Timeline of events during experimental sessions. VAS= visual analogue scale; CTRL= control session; END = 30-min moderate-intensity continuous exercise at 65%  $VO_{2max}$ ; T1= Breakfast; T2= Pre-exercise; T3= Post-exercise; T4= Recovery

**Table 3.2: Absolute GLP-1 concentration (pmol/mL) during endurance cycling and rest in men and women (mean  $\pm$  S.D.)**

Session	Male (n=10)			Female (n=9)		
	Pre-exercise*	Post-Exercise* ^	Recovery ^	Pre-exercise	Post-exercise	Recovery
CTRL	24.9 $\pm$ 6.1	29.6 $\pm$ 8.5	23.5 $\pm$ 6.6	30.9 $\pm$ 13.6	26.8 $\pm$ 15.4	21 $\pm$ 11.9
END	26.6 $\pm$ 10.0	32.8 $\pm$ 10.0	26.4 $\pm$ 9.5	22.2 $\pm$ 12.8	30.4 $\pm$ 15.7	26 $\pm$ 14.7

CTRL: resting control, END: continuous endurance cycling at 65%  $\text{VO}_{2\text{max}}$ ; \*main effect of time in men: post-exercise > pre-exercise ( $p=0.002$ ); ^ main effect of time in men: post-exercise > recovery ( $p<0.05$ )

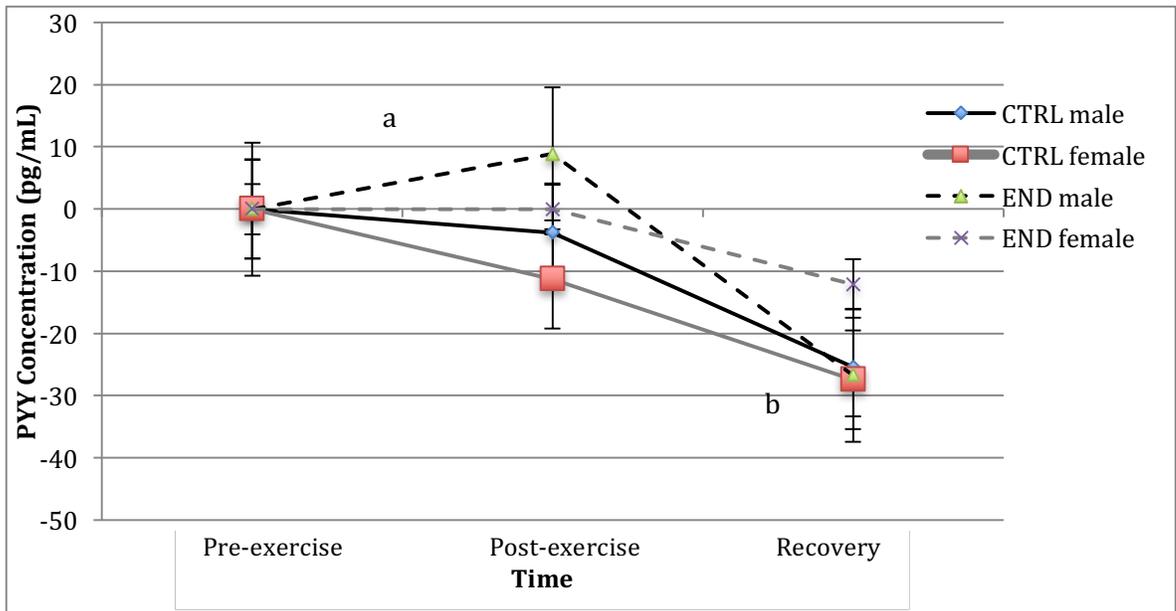


*Figure 3.2.* Change in GLP-1 concentration (mean ± S.E.) in experimental sessions (CTRL= control; END= moderate-intensity continuous exercise at 65%  $VO_{2max}$ ), across time. Values are presented as concentration change from baseline. <sup>a</sup> Session X sex interaction: Female CTRL < Male CTRL ( $p < 0.05$ ).

**Table 3.3: Absolute PYY concentration (pg/mL) in response to endurance cycling in men and women (mean  $\pm$  S.D.)**

Session	Male (n=10)			Female (n=9)		
	Pre-exercise*	Post-exercise ^	Recovery *	Pre-exercise	Post-exercise	Recovery
CTRL	107.0 $\pm$ 17.0	103.2 $\pm$ 12.3	81.5 $\pm$ 12.1	123.9 $\pm$ 38.3	112.6 $\pm$ 32.3	96.4 $\pm$ 31.2
END	123.9 $\pm$ 29.2	132.8 $\pm$ 36.9	97.2 $\pm$ 39.8	107 $\pm$ 59.6	106.9 $\pm$ 59.6	93.9 $\pm$ 60.6

CTRL: resting control, END: continuous endurance cycling at 65%  $VO_{2max}$ ; \*main effect of time in men: pre-exercise > recovery ( $p < 0.001$ ); ^ main effect of time in men: post-exercise > recovery ( $p < 0.001$ ).



*Figure 3.3.* Change in PYY concentration (mean  $\pm$  S.E.) in experimental sessions (CTRL= control; END = moderate-intensity continuous exercise at 65%  $\text{VO}_{2\text{max}}$ ) across time. Values are presented as concentration change from baseline. <sup>a</sup> Main effect of session: END > CTRL ( $p < 0.05$ ); <sup>b</sup> Main effect of time: Recovery < Post-exercise ( $p < 0.05$ ).

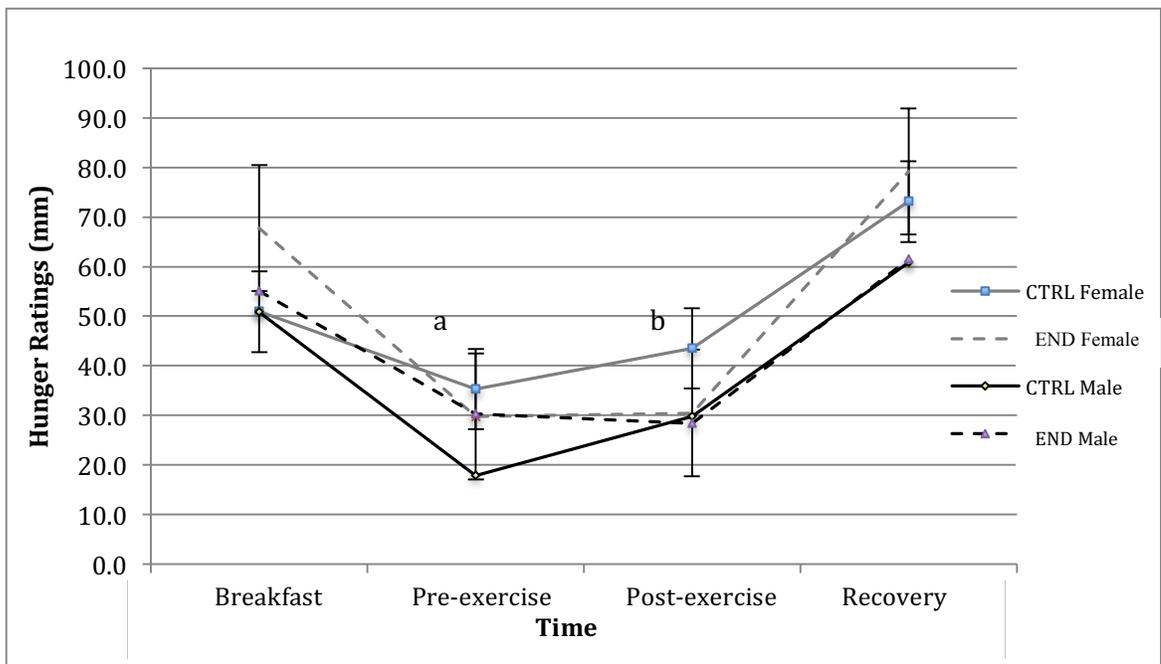


Figure 3.4. Subjective hunger ratings (mean  $\pm$  S.E.) during control (CTRL) and continuous END cycling at 65%  $VO_{2max}$  (END) sessions. Scores are represented as values along a scale of 1-100. <sup>a</sup> Pre-exercise < breakfast and recovery ( $p < 0.05$ ), <sup>b</sup> Post-exercise < breakfast and recovery ( $p < 0.05$ )

## **Chapter 4: General Discussion and Conclusion**

Weight gain and fat accumulation occur when the body is in a chronic state of positive EB. Much research has been performed to identify the most effective method of eliciting weight loss by producing a negative EB through diet and exercise. Recently, SIT exercise has been reported as an exercise technique by which one can efficiently lose body fat by performing short bouts of “all-out” effort, separated by periods of active recovery (Boutcher, 2011). Researchers have found that SIT protocols can be as effective in decreasing body fat as traditional END exercise while taking less time, though this response may differ in men and women (Henderson et al., 2008; MacPherson, Hazell, Olver, Paterson & Lemon, 2011; Trapp, Chisholm, Freund & Boutcher, 2008; Tremblay, Simoneau & Bouchard, 1994). Negative EB from exercise alone tends to be less effective in reducing body fat than diet only interventions and diet and exercise combined, with suggestions that the efficacy is even less in women (Donnelly et al., 2003; Donnelly & Smith, 2005; Hall et al. 2012; King et al. 2007; Oscai, Mole & Holloszy, 1971). It has been suggested that exercise may stimulate a compensatory increase in EI to match EE, reducing or even eliminating the energy deficit and potential for fat loss promoted by exercise (Hagobian et al., 2009). Additionally, females may be more likely to precisely match or exceed EI to EE following exercise, perhaps as a physiological protection against negative EB for protection of reproductive function, making fat loss from exercise less likely (Henderson et al., 2008). Exercise research utilizing female participants is limited and the effect of exercise on hunger and ER hormones in women is largely unknown. The overall goal of this work was to determine the effect of exercise intensity on satiety hormones and hunger in women and to compare this response between men and women following continuous endurance exercise.

## Exercise Intensity in Females

This research was the first to study and compare the effect of exercise intensity on satiety hormones GLP-1 and PYY in women. With SIT becoming more popular due to recent evidence proposing the comparable effects on body composition of this training style to traditional END exercise, we set out to examine whether SIT differentially affected satiety hormones and perceptions of hunger compared to END exercise in women. Exercise caused an increase in circulating GLP-1 concentration compared to the resting control, regardless of intensity. We found no effect of exercise on circulating PYY concentration or hunger compared to rest. Although there is no past research of this nature in women, our results are consistent with similar research comparing the response of GLP-1 and PYY to steady state exercise and SIT in men (Howe, Hand & Manore, 2014; Martins, Morgan & Truby, 2008). In contrast, however, hunger was decreased in men post-exercise - even with endurance exercise, with a greater suppression of hunger following SIT (Deighton, Karra, Batterham & Stensel, 2013), where we found no change perceptions of hunger with exercise at either intensity. Our findings suggest that women do not experience the same appetite-suppressing effect from exercise as men, regardless of intensity. A discussion on the differences in the effect of exercise on hunger and these hormones between men and women will follow this discussion of exercise intensity and satiety hormones.

Currently, of the available literature examining the effect of acute aerobic exercise on subjective hunger and appetite regulating hormones, only two studies specified a population of exercise-trained women and only one of those studies examined the effect of exercise on appetite hormones (Larson-Meyer et al., 2012). Larson-Meyer et al. (2012) utilized exercise bouts that were 60-min long at 70%  $VO_{2max}$  compared to the 27- and 30-

min bouts in our SIT and END sessions. Our results for GLP-1 and hunger are consistent with what was reported in by Larson-Meyer et al. (2012), however the response that we observed in PYY differed from their finding of increased PYY concentration post-exercise compared to rest. Exercise duration may be one reason for the difference in our PYY result.

There was no difference in the perception of hunger or concentration of satiety hormones between SIT and END exercise in women. Although there are a multitude of factors involved in fat loss from exercise, acute-exercise induced changes in PYY and GLP-1 do not appear to differ between SIT and END cycling and are not expected to be largely involved in the effectiveness of these intensities of exercise for fat loss. Our results also do not indicate the likelihood for increased compensatory EI resulting from changes in GLP-1 and PYY following one form of exercise over the other, however, EI is affected by factors other than physiological hunger. As such, we saw no reason to believe that fat loss would be greater from one exercise method over the other due to changes in PYY, GLP-1, and hunger, and therefore exercise prescription for fat loss should be centered on an individual's preferred activity type rather than its proposed efficacy for body fat reduction. Although SIT has produced similar changes in body composition to END exercise in past research, it has been suggested that the intense nature of this activity leads to greater rates of injury and dropout (Boutcher, 2011). To prescribe effective interventions for fat loss, personal preference and abilities must be considered. For longevity of exercise involvement, an individual should choose to engage in an exercise routine that is challenging, enjoyable and preventative of injury, which may involve the inclusion of both exercise techniques.

## **Sex Differences in Appetite Hormone Response**

This was the first study known to compare the response of satiety hormones to END cycling in men and women. With many people engaging in aerobic exercise programs for fat loss, and the suggestion that women are less likely to experience fat loss from exercise because of compensatory post-exercise EI, it is important to examine whether there are sex differences in the factors that influence EB and fat loss. Since less exercise research is performed on women due to the complexity of controlling for menstrual cycle, little is known about the response of exercise on appetite hormones and hunger in women. We examined and compared the effect of END cycling on subjective hunger and satiety hormones GLP-1 and PYY in men and women, in the follicular phase of the menstrual cycle (day 1-10). Phase of menstrual cycle was monitored to assure that the response of appetite regulating hormones was not being influenced by natural fluctuations in estrogen levels that occur across the menstrual cycle. Additionally, onset of menstruation, indicating the start of the follicular phase, allowed for easy tracking of participants' menstrual phase without the need for blood sampling or daily monitoring of basal body temperature. The effect of exercise on these hormones in males was consistent with current research, indicating elevated PYY and GLP-1 concentration in response to endurance exercise (Broom, Stensel, Bishop, Burns, & Miyashita, 2007; Deighton et al., 2013; Kawano et al., 2013; King et al., 2011; Shorten, Wallman & Guelfi, 2009; Vantanser-Ozen, Tiryaki-Sonmez, Bugdayci & Ozen 2011). In comparison, the GLP-1 response in females was consistent with findings from the available literature, however, the response of PYY in females differed, as exercise in our study did not elicit a significant increase in concentration compared to rest, as in other studies (Ueda et al., 2009; O'Conner, Johnston, Buchanan, Boreham, Trinick & Riddoch, 1995). We found no

significant difference between men and women in the response of these hormones to exercise. This finding contradicts the assumption that females would have lower levels of these satiety hormones and increased hunger, based on the theory that women are more likely to experience a compensatory increase in EI post-exercise to match the EE of exercise. There is no known literature comparing the effect of endurance exercise on satiety hormones in men and women and therefore we are unable to comment on the consistency of our findings with current literature.

Although literature comparing the effect of exercise intensity on these satiety hormones in men and women is unavailable, assumptions can be made based on comparisons with past research in males and the results obtained in our first study on females. As mentioned earlier, exercise can have a satiating effect in males, with greater appetite suppression following higher intensity exercise (Deighton et al., 2013). This is supported by twelve other published, peer-reviewed studies that examined the effect of exercise on appetite post-exercise in men. We did not observe a satiating effect of exercise in women following either END or SIT protocols, which is consistent with the previous two studies examining this effect in women following 60-min of treadmill walking at 70%  $VO_{2max}$  (Larson-Meyer et al., 2012; Pomerleau, Imbeault, Parker & Doucet, 2004). This sex difference in the suppression of hunger following exercise may help to explain the differences observed in past research; that fat loss is greater in men compared to women as a result of exercise training. While we did not observe changes in satiety hormones PYY and GLP-1 that would indicate the potential for increased hunger and compensatory EI post-exercise in women, it is important to note that beyond these hormones there are various psychological and physiological factors that can collectively influence hunger and EI post-exercise (Martins, Morgan, Bloom & Robertson, 2007).

Additionally, while we did not see a difference in men and women's hunger following exercise in our second study, the potential for this difference has been clearly outlined above with the contrasting results obtained in research with women compared to the larger body of available evidence in men. There appears to be contradictory evidence in the literature on sex differences in the response to exercise, so further research is required.

As we have not observed a significant sex differences in the effect of acute aerobic exercise on satiety hormones and hunger, there is no reason to believe that, based on our findings, exercise prescription for fat-loss should differ between men and women. As stated above, there are a variety of physiological and psychological factors that influence overall appetite and EI post-exercise that need to be considered when pursuing fat-loss (Martins et al., 2007). Finally, enjoyment and risk of injury are critical factors that need to be considered in the pursuit of fat loss from exercise. For longevity of participation, a person seeking fat loss, whether male or female, should engage in an exercise program that they enjoy and are likely to stick with for the long-term. Finally, the preferred method must be able to be sustained over time without injury or burnout, which may require the integration of both protocols.

### **Strengths and Limitations**

A major strength of this study was the use of a randomized crossover design that required completion of all sessions by each participant and allowed for participants to act as their own control. Another strength was controlling for phase of menstrual cycle in scheduling sessions for female participants. By testing participants during the early follicular phase, between days 1 and 10 of their menstrual cycle, we minimized the

likelihood that fluctuations in appetite hormones occurred due to associated changes with sex hormones.

A limitation of this study was the small number of subjects recruited, lowering statistical power. In addition, our study was limited in that we only studied satiety hormones and did not include any measurement of the appetite-stimulating hormone, acylated ghrelin. Furthermore, our study measured concentrations of total PYY and GLP-1. Future studies would benefit from adapting the hormonal profile measured to include: acylated ghrelin, PYY<sub>3-36</sub>, which has been shown to play a major role in appetite control (Chelikani, Haver & Reidelberger, 2004) and GLP-1<sub>9-36</sub>, which is the major form of GLP-1 post-prandially (Vahl, Paty, Fuller, Prigeon & D'Allesio, 2003). Future studies should also note that beyond these appetite hormones, psychological (motivation to eat, palatability and dietary restraint) and physiological (other appetite-regulating hormones), along with other body factors (body weight, training status and sex), collectively influence hunger and should be considered in their research.

Another possible limitation was that total EE was not measured throughout the sessions. Discrepancies in EB are likely to influence the circulating levels of these hormones. Further research should monitor total EE, ending subsequent sessions once EE has been matched. Additionally, participants were asked to replicate the same diet in the 24-h preceding each session, however this was not strictly monitored and it is possible that some participants did not strictly adhere to these guidelines. Alterations in meal composition in the day prior to hormone testing could affect EB and therefore the circulating levels of these satiety hormones. Finally, although validated, it is possible that the hunger VAS utilized was not sensitive or specific enough to identify accurate changes in hunger. Future research should utilize additional measures of hunger, including a more

complex combination of VAS allowing for the calculation of a composite hunger score (Alajmi et al., 2016), having participants indicate additional measures along subsequent VAS anchored on either end with extremes (i.e. Do you feel hungry? How hungry?; Do you feel full? How full?; Are you satisfied? How satisfied?; Would you like to eat more? How much more?; Are you thirsty? How thirsty?).

### **Conclusion**

The results of this thesis project demonstrate that exercise influences appetite regulating hormones, but that these changes do not differ between exercise intensities nor do the results following endurance exercise differ between men and women. The influence of exercise on satiety hormones GLP-1 and PYY does not suggest the potential for an increased perception of hunger following exercise nor does it suggest that one exercise intensity may increase satiety hormones more than the other. Following exercise, the increase in GLP-1 concentration did not appear to be sufficient enough to result in a suppression of hunger. A longer period of exercise training would be needed to fully examine the long-term effect of exercise, both END and SIT, on these hormones and their effect on appetite. The link between exercise intensity, appetite regulating hormones, and hunger in women remains to be fully elucidated.

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## Appendices

Informed Consent

Participant Questionnaire

Physical Activity Readiness Questionnaire (PAR-Q+)

## Informed Consent

### **CONSENT TO PARTICIPATE IN RESEARCH** **LETTER OF INFORMATION**

Date:

Title of Study: Examining the effects of exercise intensity on appetite-related hormones and energy expenditure

Dear \_\_\_\_\_:

You are being invited to participate in a research study conducted by Jennifer L. Copeland (PhD), Tom J. Hazell (PhD), and Matt Schmale (MSc) from the Exercise Nutrition and Health Research Laboratory in the Department of Kinesiology and Physical Education at the University of Lethbridge.

#### PURPOSE OF STUDY

The primary purpose of the study is to determine if the performance of cycling exercise at different intensities has an influence on the blood plasma concentrations of appetite-related hormones. The secondary purpose of the study is to determine if the performance of cycling exercise at different intensities influences energy expenditure over the following 48-hr, assessed using an accelerometer.

#### PROCEDURES

Before you participate, you will be asked to complete a Physical Activity Health Survey Questionnaire (PAR-Q). The research will take approximately 5 weeks to complete. During this time, we will require you to visit the Exercise Nutrition and Health Research Laboratory (PE248) for a familiarization session and fitness assessment (~1-hr) and 4 trial sessions (~3-hr). During the fitness assessment, you will be required to undergo skinfold measurements to determine body composition and perform a cycling maximal oxygen consumption ( $VO_{2max}$ ) test. This test will have you cycle against a constantly increasing workload until exhaustion. In the 24-hr previous to your first trial session, you will record a food diary to track energy intake and wear an accelerometer to track energy expenditure. On trial days, you will enter the laboratory in a fasting state at 8:00 a.m. where you will be fed a standardized breakfast. Following 1-hr (~9:00 a.m.), a researcher will draw 12-mL of whole blood from an antecubital vein (inside of the elbow) and you will begin your exercise session. Exercise sessions will have you 1) cycle at 85%  $VO_{2max}$  for 30-min, 2) cycle at 65%  $VO_{2max}$  for 30-min, 3) cycle for 6 “all-out” 30-s efforts each separated by 4-min of active recovery, or 4) remain seated in the lab for 30-min. Each of these exercise sessions will be performed once during the study, and will be selected at random upon entering the laboratory on the morning of the trial session. Immediately following the exercise session (~9:30 a.m.), another 12-mL of whole blood will be drawn from a separate antecubital vein, and you will remain seated

quietly in the laboratory for an additional 90-min. During this time, you may read, study, or work on homework, and a final 12-mL of whole blood will be drawn at its end (~11:00 a.m.). In the 48-hr following each trial session, you will continue wearing the accelerometer, at which point we ask it be returned.

#### POTENTIAL RISKS AND DISCOMFORTS

There is a possibility of mild muscle soreness and/or fatigue typical of an exercise session. You may feel discomfort due to the intensity of the training or performance tasks typical of strenuous physical exertion, as you are required to perform certain exercise sessions and the testing session with maximal effort. There is possibility of slight discomfort during blood draws as well as injury due to fainting.

#### CONFIDENTIALITY

Only researchers associated with this study will have access to identifying information. The research assistants associated with the study will also sign confidentiality agreements. This will ensure that any information that is obtained in connection with this study that can identify you will remain confidential. This information will be collected on a master list that will be kept in a password-protected file accessible only to the study investigators. All data will be collapsed before results are printed (only group averages and variability). All participants will be assigned an arbitrary number to ensure anonymity. Mean data will be stored in a password protected file for comparison with future studies. Raw data will not be released to any other parties.

#### PARTICIPATION AND WITHDRAWAL

Your participation in this research study is completely voluntary. You may withdraw at any time without consequence. If you are a student who chooses to withdraw, it will not affect your status at the University of Lethbridge. You may also refuse to answer any questions you feel are inappropriate and still remain in the study. The investigators may withdraw you from the study if circumstances arise which warrant doing so.

#### FEEDBACK OF THE RESULTS OF THIS STUDY

The results from this study will be reported in general terms in the form of speech or writing that may be referenced 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 a reputable scientific journal upon completion of the research. The information published in a journal or subsequent studies will not identify you in any way. Copies of such articles will be available upon request.

#### SUBSEQUENT USE OF DATA

These data may be used in subsequent studies, but the data will have no personal identifiers.

You will receive a copy of the consent form after it has been signed. You do not waive any legal rights by signing the consent form.

#### RECRUITMENT IN FUTURE STUDIES

If you check the box below, you consent to be contacted regarding potential participation in future studies in the Exercise Nutrition and Health Research Laboratory. We will contact you with information and the option to participate if you choose.

This letter is yours to keep. If you have any questions about this research project, feel free to call us:

Matt Schmale (403) 795-7979

Dr. Jennifer Copeland (403) 317-2804

Dr. Tom Hazell (519) 884-1970 ext. 3048

Further, if you have any questions about the conduct of this study or your rights as a participant, you may contact the Office of Research Ethics at the University of Lethbridge at (403) 329-2747.

Sincerely,

Dr. Jennifer Copeland ([jennifer.copeland@uleth.ca](mailto:jennifer.copeland@uleth.ca))

Dr. Tom Hazell ([thazell@wlu.ca](mailto:thazell@wlu.ca))

Matt Schmale ([matt.schmale@uleth.ca](mailto:matt.schmale@uleth.ca))

Title of Study: Examining the effects of exercise intensity on appetite-related hormones and energy expenditure

Consent Statement

Principal Investigators: Dr. Jennifer Copeland and Dr. Tom Hazell

Master's Research Assistant: Matt Schmale

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: \_\_\_\_\_

# Participant Questionnaire



## RESEARCH PARTICIPANT QUESTIONNAIRE

Title of Study: Examining the effects of exercise intensity on appetite-related hormones and energy expenditure

NAME (please print): \_\_\_\_\_ Age: \_\_\_\_\_

SEX: \_\_\_\_\_ DATE OF BIRTH (MM/DD/YYYY): \_\_\_\_\_

Please respond to the following questions with a Y or N response. If more information is needed, please provide it in the space provided:

Do you consider yourself a professional or recreational athlete? \_\_\_\_\_

If yes, please provide details: \_\_\_\_\_

Do you regularly (at least 3 times per week) perform vigorous exercise? \_\_\_\_\_

If yes, please provide details: \_\_\_\_\_

Do you feel comfortable that you can complete high-intensity, exhaustive workouts of 20-30 minutes? \_\_\_\_\_

Have you completed the Physical Activity Readiness Questionnaire (PAR-Q)? \_\_\_\_\_

Do you have a history of drug or alcohol abuse within the last 2 years? \_\_\_\_\_

Do you have a history of heart disease? \_\_\_\_\_

If yes, please provide details: \_\_\_\_\_

Have ever been diagnosed with anemia? \_\_\_\_\_

If yes, please provide details: \_\_\_\_\_

Have you ever been diagnosed with type 1 or type 2 diabetes? \_\_\_\_\_ Other metabolic disease? \_\_\_\_\_

If yes, please provide details: \_\_\_\_\_

# Par-Q+

CSEP approved Sept 12 2011 version

## PAR-Q+

### The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

#### SECTION 1 - GENERAL HEALTH

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.		YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	<input type="checkbox"/>	<input type="checkbox"/>
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	<input type="checkbox"/>	<input type="checkbox"/>
5.	Are you currently taking prescribed medications for a chronic medical condition?	<input type="checkbox"/>	<input type="checkbox"/>
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	<input type="checkbox"/>	<input type="checkbox"/>
7.	Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

If you answered NO to all of the questions above, you are cleared for physical activity.



Go to Section 3 to sign the form. You do not need to complete Section 2.

- › Start becoming much more physically active – start slowly and build up gradually.
- › Follow the Canadian Physical Activity Guidelines for your age ([www.csep.ca/guidelines](http://www.csep.ca/guidelines)).
- › You may take part in a health and fitness appraisal.
- › If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- › If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.



If you answered YES to one or more of the questions above, please GO TO SECTION 2.



Delay becoming more active if:

- › You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- › You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- › Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.