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THE ASSOCIATION BETWEEN WEIGHT LOSS INDUCED SUPPRESSION OF LH PULSATILITY  
AND METABOLIC HORMONES

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## ABSTRACT

It has been demonstrated that ghrelin and cortisol are elevated in women with exercise-associated menstrual disturbances (EAMD). Additionally, it is suggested that ghrelin is a key factor contributing to the suppression of luteinizing hormone (LH) pulse frequency, a proxy indicator of menstrual function. However, the mechanism by which ghrelin modulates LH pulsatility is unknown. The purpose of this study was to test the hypothesis that cortisol is a potential intermediary in the association between ghrelin and LH in normal weight, sedentary women subsequent to a controlled feeding and exercise intervention designed to induce an energy deficit. This study was part of a larger study designed to assess the endocrine and reproductive changes in women in response to an intervention controlling food intake and supervised exercise (5 days/week). Subjects (5 sedentary controls [C], 16 exercising energy deficit [Edef]) were studied at baseline (BL) and subsequent to (Post) the 3-month intervention. Blood samples were obtained every 10 minutes for 24 hours and assayed using radioimmunoassay for total ghrelin. Immulite was used to measure serum concentrations of LH and ELISA was utilized to measure concentrations of 24-hour urinary cortisol. Statistical analyses included paired T-tests, ANOVA and stepwise linear regression. Subjects in the Edef group lost a significant amount of body weight ( $58.4 \pm 1.1$  kg to  $55.3 \pm 1.2$  kg,  $p < 0.001$ ), body fat percentage ( $28.3 \pm 1.2$  to  $24.6 \pm 1.2$ ,  $p < 0.001$ ), body fat mass ( $16.6 \pm 0.8$  kg to  $13.7 \pm 0.9$  kg,  $p < 0.001$ ), and increased VO<sub>2</sub> max ( $37.1 \pm 1.1$  ml/kg/min to  $43.0 \pm 1.5$  ml/kg/min,  $p = 0.001$ ) whereas C subjects remained weight stable ( $p = 0.146$ ). Ghrelin AUC ( $28903 \pm 2739$  to  $34799 \pm 3382$ ,  $p = 0.002$ ), 24-hour mean ( $1216.9 \pm 126.3$  to  $1457.8 \pm 150.3$ ,  $p = 0.002$ ) and peak ( $1602.4 \pm 153.2$  to  $2007.2 \pm 214.7$ ,  $p = 0.003$ ) as well as 24-hour cortisol ( $40.7 \pm 3.4$  to  $57.3 \pm 5.9$ ,  $p =$

0.018) significantly increased whereas LH pulse frequency ( $0.81 \pm 0.06$  to  $0.63 \pm 0.08$ ,  $p = 0.047$ ) significantly decreased from BL to post in the Edef group while there was no change exhibited in the C group in any ghrelin parameter, 24-hour cortisol or LH pulse frequency ( $p > 0.05$ ). In the Edef group, the change in ghrelin AUC ( $p < 0.001$ ) and the change in body weight ( $p = 0.03$ ) significantly predicted the change in 24-hour cortisol. Also observed in the Edef group, the change in ghrelin 24-hour mean ( $p = 0.01$ ) was the only significant predictor of the change in LH pulse frequency. Thus, the association between cortisol and LH was not demonstrated in this study. In conclusion, cortisol may be also be involved in the suppression of the HPO axis, but is likely not an intermediary in the association between ghrelin and LH.

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## CHAPTER 1

### LITERATURE REVIEW

#### Energy Balance and Reproduction

In response to an energy deficit where energy intake does not adequately meet the needs of energy expenditure, two body systems can be forgone to conserve energy: growth and reproduction (Wade, Schneider, & Li, 1996). The reproductive axis, a system requiring energy but not vital to survival, appears to adapt during times of energy deficiency and becomes suppressed by a currently unknown mechanism. Reproductive suppression, secondary to an energy deficit, has been associated with several adverse complications such as decrements in bone and cardiovascular health (De Souza & Williams, 2004). Also, several aberrations occur in endocrine hormones in response to an energy deficit, including those directly, such as suppressed luteinizing hormone pulsatility (Loucks & Thuma, 2003), and indirectly, such as elevated concentrations of ghrelin (Scheid, De Souza, Leidy, & Williams, 2011) and cortisol (Misra, et al., 2004), involved in the control of the menstrual cycle.

Recent research has demonstrated a high incidence of menstrual disturbances in exercising women ranging from subtle disturbances like luteal phase defects (luteal phase length of <10 days) or inadequate luteal phase where progesterone secretion is suppressed to functional hypothalamic amenorrhea (FHA; the loss of menses for  $\geq 90$  days) in exercising women (De Souza, et al., 2007). These exercise-associated menstrual disturbances (EAMD) are not due to the stress of exercise per se, but likely secondary to an energy deficit which induces the adaptive mechanisms that are believed to conserve energy (Williams, et al., 1995). Evidence to this effect was first demonstrated by Williams et al. in

1995 (Williams, 1995). Eight Rhesus monkeys were exercised to induce an energy deficit and induce amenorrhea. Four monkeys were provided increased caloric intake while maintaining exercise energy expenditure and subsequently resumed menses.

### **Endocrine Control of the Menstrual Cycle**

A healthy menstrual cycle is regulated by a number of endocrine feedback mechanisms via a specific hormone pathway known as the hypothalamic-pituitary-ovarian (HPO) axis. Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates release of two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the anterior pituitary gland. LH and FSH bind to receptors on the ovaries stimulating the growth of the follicle within the ovary as well as synthesis and secretion of estrogen. Estrogen is a hormone that negatively feeds back to the hypothalamus and pituitary gland to inhibit LH and FSH release. Interestingly though, as the follicle grows and produces more estrogen, a mid-cycle surge occurs and estrogen becomes a positive feed forward stimulus for a mid-cycle LH surge, which causes ovulation (Bliss, Navratil, Xie, & Roberson, 2010).

### **Luteinizing Hormone**

Spontaneous GnRH pulses from neurons in the hypothalamus stimulate the synthesis and pulsatile release of LH from the anterior pituitary gland. The release of LH in pulses is critical for proper ovarian estrogen production and function of the reproductive axis. In response to an energy deficit, it is hypothesized that GnRH pulsatility is suppressed and as a consequence, LH pulsatility is disturbed, inducing the manifestation of menstrual cycle disturbances (Wade & Jones, 2004). This has been demonstrated in patients with anorexia nervosa (AN) where the 24-hour profile of GnRH and LH pulsatility were suppressed (Katz, Boyar, Roffwarg, Hellman, & Weiner, 1978). In addition, healthy

ovulatory women have been shown to respond to a short term energy deficit of only five days by manifesting with suppressed LH pulsatility (Loucks & Thuma, 2003).

### **Cortisol**

Cortisol is a steroid hormone that is synthesized and secreted from the zona fasciculata of the adrenal cortex in response to metabolic and/or psychological stress. The hypothalamic-pituitary-adrenal (HPA) axis is responsible for regulating cortisol release. Corticotrophin-releasing hormone (CRH) is secreted from the hypothalamus and stimulates the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH). ACTH binds to receptors on the adrenal cortex, which in turn stimulates the release of cortisol. Cortisol's primary functions are to increase blood sugar through gluconeogenesis (glucose production from sources other than carbohydrate), suppress the immune system, and aid in macronutrient metabolism (Milosević, et al., 2010). The HPA axis is stimulated in response to fasting (Dallman, et al., 1999) whereas circulating concentrations of cortisol are suppressed in response to constant feeding (Saito, Nishimura, & Kato, 1989). Thus, cortisol not only responds to stress, but may also play an important role in energy balance.

The hormonal profile of circulating cortisol appears to be altered in response to an energy deficit in women. dos Santos et al. (2007) found an absence of the cortisol circadian rhythm in women with AN. Interestingly the authors reported that the circadian rhythm of cortisol was lost, but subjects generally had elevated salivary cortisol levels in comparison to the healthy controls. Additionally, Misra et al. (Misra, et al., 2004) observed elevated 24-hour urinary and serum cortisol concentrations in women with AN. However, in this study, the authors observed a preservation of the circadian rhythm of cortisol. Suh et al. (Suh, et

al., 1988) also reported this phenomenon of chronically elevated cortisol in women with FHA.

## **Stress**

Psychological stress is often a contributing factor in relation to restrictive eating patterns (disordered eating and eating disorders) typically observed in women with FHA (Marcus, Loucks, & Berga, 2001). Though it has been previously demonstrated that menstrual disturbances in exercising women is likely due to an energy deficit and not the physical stress of the exercise itself (Williams, et al., 1995), the role of psychological stress may also impact the reproductive axis. In mammalian models, females who are socially dominant, which is an indication of psychological stress, have been shown to have luteal phase defects and hypoestrogenism (Adams, Kaplan, & Koritnik, 1985). One will experience stress when environmental influences test or surpass the resources available to them (Cohen, Kessler, & Gordon, 1995). Specific physiological responses are up-regulated in response to stress; one of which is the activation of the HPA axis. Activation of this pathway leads to subsequent cortisol release. Elevations in serum concentrations of cortisol have been associated with hypertension (Krakoff, 1988), decreased bone mineral density (Ding, Sheckter, Drinkwater, Soules, & Bremner, 1988) and increased abdominal fat deposition (Rebuffé-Scrive, Walsh, McEwen, & Rodin, 1992). Additionally, elevated concentrations of circulating cortisol have been observed in women with EAMD and may be a contributing factor in the suppression of the reproductive axis.

### **Cortisol and LH Pulsatility**

As demonstrated by Suh et al. (1988), cortisol concentrations were elevated in women with FHA. Additionally, one study in a bovine model in 1983 observed that in vitro cortisol administration to pituitary cells inhibited LH release (Padmanabhan, Keech, & Convey, 1983). These observations may be explained by inhibition of GnRH secretion by elevations in CRH (Chen, O'Byrne, Chiappini, Hotchkiss, & Knobil, 1992) and the presence of glucocorticoid receptors on GnRH neurons in the hypothalamus (Chandran, et al., 1994). In a more recent study, the authors concluded that high baseline serum cortisol significantly predicted lack of menstrual resumption in women with AN (Arimura, et al., 2010). Therefore, cortisol likely plays a role in the suppression of the HPO axis through suppression of LH pulsatility.

### **Ghrelin**

Ghrelin is a growth hormone (GH) secretagogue that is primarily released from the stomach. Subsequent to its discovery in the late 1990's (Kojima, et al., 1999), ghrelin demonstrated orexigenic properties (Wren, et al., 2000), stimulating energy intake and being termed the only known hunger hormone to date. Ghrelin is secreted from endocrine cells in the stomach (Date, et al., 2000), increases in the fasted state, peaking just before a meal and declines subsequent to energy intake (Leidy, et al., 2004). Circulating concentrations of ghrelin are altered in humans in response to changes in chronic energy balance. In obese individuals, suppressed concentrations of ghrelin have been reported (Tschöp, et al., 2001) and in individuals suffering from anorexia nervosa (AN), increased concentrations of ghrelin have been observed (Otto, et al., 2001). Additionally, during times of fasting and subsequent weight loss, ghrelin concentrations are also elevated in

both obese (Cummings, et al., 2002) and lean (Leidy, Dougherty, Frye, Duke, & Williams, 2007) individuals. Recently, it has been demonstrated that in women with EAMD, circulating concentrations of ghrelin are elevated as well (Scheid, De Souza, Leidy, & Williams, 2011).

### **Ghrelin and LH Pulsatility**

In addition to its role in acute and chronic energy balance, ghrelin may also play a role in suppressing the endocrine regulation of the HPO axis. In one study, a decrease was observed in LH pulse frequency in response to a 5 hour ghrelin infusion in ovariectomized rhesus monkeys (Vulliémoz, et al., 2004). A recently published article by Kluge et al. (2007) demonstrated this in humans, specifically showing a direct relationship between the administration of ghrelin and decreases in both LH and FSH. However, ghrelin receptors are not expressed on the GnRH neurons in the hypothalamus (Guan, et al., 1997), suggesting that ghrelin's role in the suppression of LH pulsatility is likely indirect.

### **Cortisol and Ghrelin Interactions**

It has been demonstrated that both ghrelin and cortisol are elevated in women with menstrual cycle disturbances secondary to an energy deficit. Ghrelin, in addition to being a GH secretagogue and hunger hormone, may be involved in the modulation of ACTH secretion (Misra & Klibanski, 2010). In rats, ghrelin has been shown to directly stimulate the HPA axis by stimulating CRH (Mozid, et al., 2003). Vulliemoz et al. (Vulliémoz, et al., 2004) demonstrated that ghrelin infusion significantly decreased LH pulse frequency and stimulated the release of cortisol. Authors concluded that ghrelin may be involved in activation of the HPA axis, but that the relevance of this activation to in the inhibition of GnRH pulsatility remained to be determined. From the culmination of these findings, it may

be inferred that elevated concentrations of cortisol may be a contributing factor to increased circulating concentrations of ghrelin; both of which are endocrine manifestations typically observed in women with FHA. Additionally, the suppression of LH pulsatility as a result of elevated circulating concentrations of ghrelin may be attributable in part to elevations in circulating concentrations of cortisol.

## CHAPTER 2

### INTRODUCTION

In response to an energy deficit where energy intake does not adequately meet the needs of energy expenditure, two body systems can be forgone to conserve energy: growth and reproduction (Wade, Schneider, & Li, 1996). The reproductive axis, a system requiring energy but not vital to survival, appears to adapt during times of energy deficiency and becomes suppressed by a currently unknown mechanism. Reproductive suppression has been associated with several adverse complications such as decrements in bone and cardiovascular health (De Souza & Williams, 2004). Recent research has demonstrated a high incidence of menstrual disturbances like FHA in exercising women (De Souza, et al., 2007). The development of EAMD is not due to the stress of exercise per se, but likely secondary to an energy deficit which induces the adaptive mechanisms that are believed to conserve energy (Williams, et al., 1995).

It has been demonstrated that endocrine hormones outside of the HPO axis, namely cortisol (Padmanabhan, Keech, & Convey, 1983), the stress hormone, and ghrelin (Vulliémoz, et al., 2004), a gastrointestinal hormone, may be involved in the modulation of the endocrine regulation of the menstrual cycle. Circulating concentrations of cortisol (Misra, et al., 2004) and ghrelin (Scheid, De Souza, Leidy, & Williams, 2011) have been shown to be elevated in women with EAMD and thus may be involved in the manifestation of menstrual disturbances.

Cortisol is a steroid hormone released via the HPA axis in response to stress. Cortisol's primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in macronutrient metabolism (Milosević, et al., 2010). The

HPA axis is stimulated in response to fasting (Dallman, et al., 1999) whereas circulating levels of cortisol are suppressed in response to constant feeding (Saito, Nishimura, & Kato, 1989). Studies by dos Santos et al. (dos Santos, et al., 2007) and Misra et al. (Misra, et al., 2004) found elevations in circulating concentrations of cortisol in women with AN. Suh et al. (Suh, et al., 1988) also reported chronically elevated cortisol in women with FHA.

The role of cortisol in LH pulsatility has been suggested through the discovery of glucocorticoid receptors on GnRH neurons in the hypothalamus (Chandran, et al., 1994) and that CRH may be implicated in the suppression of GnRH release (Chen, O'Byrne, Chiappini, Hotchkiss, & Knobil, 1992). Moreover, it has been demonstrated that cortisol inhibited LH release from the pituitary gland in cows (Padmanabhan, Keech, & Convey, 1983). In a more recent study, authors concluded that high baseline serum cortisol was the only significant predictor of the lack of resumption of menses in women with AN (Arimura, et al., 2010). Consequently, cortisol may play a role in the suppression of the HPO axis and menstrual cycle disturbances through suppression of LH pulsatility.

Ghrelin is secreted from endocrine cells in the stomach (Date, et al., 2000), increases in the fasted state, peaking just before a meal and declines subsequent to energy intake (Leidy, et al., 2004). Circulating concentrations of ghrelin are altered in humans in response to changes in both acute and chronic energy balance. In obese individuals, suppressed concentrations of ghrelin have been reported (Tschöp, et al., 2001) and in individuals suffering from AN, increased concentrations of ghrelin have been observed (Otto, et al., 2001). Additionally, during times of fasting and subsequent weight loss, ghrelin concentrations are elevated in both obese (Cummings, et al., 2002) and lean (Leidy, Dougherty, Frye, Duke, & Williams, 2007) individuals. Recently, it has been demonstrated

that in women with EAMD, circulating concentrations of ghrelin are elevated as well (Scheid, De Souza, Leidy, & Williams, 2011).

In addition to its role in acute and chronic energy balance, ghrelin may also play a role in suppressing the HPO axis. In one study, a decrease was observed in LH pulse frequency in response to a 5-hour ghrelin infusion in ovariectomized rhesus monkeys (Vulliémoz, et al., 2004). A recent study by Kluge et al. (Kluge, Schüssler, Uhr, Yassouridis, & Steiger, 2007) demonstrated this in humans, specifically showing a direct association between the administration of ghrelin and decreases in both LH and FSH. However, ghrelin receptors are not expressed on the GnRH neurons in the hypothalamus suggesting that ghrelin's role in the suppression of LH pulsatility is indirect (Guan, et al., 1997).

Ghrelin and cortisol are elevated in women with menstrual cycle disturbances secondary to an energy deficit. Ghrelin may be involved in the modulation of ACTH secretion (Misra & Klibanski, 2010). In rats, ghrelin has been shown to directly stimulate the HPA axis by stimulating CRH (Mozid, et al., 2003). From this it can be inferred that elevated concentrations of cortisol may be mediated by increased circulating concentrations of ghrelin; both of which are endocrine manifestations typically observed in women with FHA. The modulation of LH pulsatility through elevations in circulating concentrations of ghrelin is likely indirect and this association may be mediated through cortisol because there are glucocorticoid receptors on GnRH neurons.

The purpose of this study was to determine if alterations in circulating concentrations of cortisol and ghrelin were associated with suppression of reproductive function in normal weight, sedentary women subsequent to a controlled feeding and exercise intervention designed to induce an energy deficit. Our hypotheses were several-

fold. First, we hypothesized that elevated concentrations of cortisol and ghrelin would be observed subsequent to weight loss in those subjects undergoing the intervention in comparison to sedentary controls. Second, we hypothesized that increased circulating concentrations of ghrelin in response to diet- and exercise-induced weight loss will be significantly associated with increases in 24-hour urinary cortisol. Finally, we hypothesized that increased 24-hour urinary cortisol will be significantly associated with suppressed LH pulsatility. The proposed mechanism by which the HPO axis is suppressed in response to an energy deficit is as such: we hypothesize that the increases in cortisol may mediate the suppressive effect of ghrelin on LH pulse frequency.

## CHAPTER 3

### MATERIALS AND METHODS

#### Study Overview and Screening

This study was part of a larger study designed to assess the endocrine and reproductive changes in women in response to an intervention controlling food intake and exercise. This intervention was implemented in healthy, sedentary women and designed to mimic exercise and restrictive eating patterns in which many young women typically engage. The subjects recruited for this study were non-smoking, non-exercising (< 1 hour/week purposeful exercise) women ages 18-30, with 15-30% body fat and a BMI between 18-25 kg/m<sup>2</sup>. Exclusion criteria included evidence or history of an eating disorder, loss/gain of a significant amount of weight ( $\pm 2.3$ kg) in the past year or use of hormonal contraceptives or medication that may alter metabolism. All subjects signed an informed consent approved by the Institutional Review Board of The Pennsylvania State University.

Subjects provided information regarding demographics, medical and menstrual history, and physical activity. Additionally, subjects completed an eating attitudes questionnaire. A fasting blood sample was taken from each participant for analysis to rule out any metabolic or endocrine dysfunction and included a basic chemistry panel and a complete blood count. A clinical psychologist interviewed each subject to assess psychological stability and eating behaviors to rule out eating disorders. A registered dietician also interviewed the subjects to discuss eating behaviors and to ensure that subjects were suitable for a controlled feeding study. Two to three ovulatory menstrual cycles were documented prior to the beginning of the study confirmed by the measurement

of mid-luteal phase serum progesterone and a mid-cycle urinary LH surge (First Response, Tambrands, Inc.).

### **Subject groupings**

Upon entering baseline, subjects were randomized into one of two groups. The control (C) group (n = 5) consisted of sedentary, non-exercising women who were to remain weight stable throughout the three month intervention. The exercising, energy deficit (Edef) group (n = 16) consisted of women who were provided fewer calories and exercised to induce an energy deficit. Meals were controlled for each group and exercise was prescribed and monitored in the Edef group. Subjects were weighed periodically throughout the intervention to confirm weight stability in the C group and weight loss in the Edef group.

### **Dietary intake during the intervention**

All meals during the intervention were prepared and provided by registered dieticians in the general clinical research center (GCRC). Three meals and a snack were prepared and carefully monitored throughout the intervention to obtain accurate estimates of caloric intake at each meal. Macronutrient composition was kept consistent among meals with 55% carbohydrate, 15% protein, and 30% fat. Any uneaten food was reweighed and calories subtracted from the total caloric content to accurately quantify intake. Subjects in the C group were provided enough calories to maintain body weight. In the Edef subjects, dietary intake was decreased by  $460 \pm 151$  kcal/day from baseline energy needs.

### **Exercise training during the intervention**

To meet the prescribed energy deficit, energy expenditure was manipulated through supervised aerobic exercise in only the Edef group. Subjects were exercised 5 days per week at 70% of their  $VO_{2max}$  until prescribed caloric expenditure was met. Calories were recorded from a Polar heart rate monitor worn during exercise bouts using the OwnCal feature on the Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland). Adjustments in exercise time were made based on the actual amount of calories expended during this workout. During non-exercise days subjects were instructed to return to their typical lifestyle.

### **Fitness and Body Composition**

All subjects were weighed periodically throughout the intervention in shorts and a T-shirt to the nearest 0.1 kg. Hydrostatic weighing was used to determine body density after correcting for residual lung volume. From this measurement, fat free mass and percent body fat were estimated using the Brozek equation (Brozek, Grande, Anderson, & Keys, 1963).

Maximal aerobic capacity was determined using indirect calorimetry during the baseline and post-intervention time-points according to previously published methods (Leidy, Dougherty, Frye, Duke, & Williams, 2007).

### **Twenty-four hour Urine Collection**

Subjects at baseline and subsequent to the three month intervention collected urine for one continuous 24 hour period. After the first morning void, start time was recorded by the subject and all voids thereafter were collected in the same container for the following 24 hours. Stop time was recorded up to or past the full 24 hour period. If samples were

collected for more than the 24-hour period, total volume was extrapolated to 24 hours. Subjects stored the sample collection container in a freezer when possible and were provided freezer packs to keep samples cool for transportation to lab. Upon arrival to the lab, samples were immediately processed, aliquoted to microcentrifuge tubes and stored at  $-20^{\circ}\text{C}$  until analysis.

### **Determination of Baseline Energy Needs**

Caloric intake required to maintain weight for each subject (baseline energy needs) was calculated based on the sum of energy expenditure from the measurement of 24-hour resting metabolic rate (RMR) and activity monitor (AM) kcal. RMR (kcal/24hr) was measured through indirect calorimetry. Subjects arrived at the laboratory subsequent to an overnight fast and having abstained from exercise for 24 hours. Subjects lay in a supine position for 45 minutes which was followed by a 45 minute RMR test. Twenty-four hour RMR was subsequently extrapolated. Twenty-four hour energy expenditure above rest was recorded via AM (RT3 accelerometer; Stayhealthy, Monrovia, CA) worn on subjects' right hip for 7 days with the exception of showering and sleeping. The prescribed diet was based on 24-hour energy expenditure, prepared by the GCRC metabolic kitchen and then provided for a 7-day calibration period during baseline. Subjects were weighed daily, and  $\pm 100$  kcal/day adjustments were made if body weight fluctuated by more than  $\pm 1$  kg. The 7-day diet was comprised of 55% carbohydrates, 30% fat, and 15% protein and totaled the amount of calories that represented one's individual energy needs.

### **Twenty-four hour repeated Blood Sampling**

All subjects were tested during the follicular phase (days 2-7) at baseline, at least one week after the calibration period, and also during the post-exercise intervention. Subjects were instructed to abstain from exercise or caffeine ingestion 24 hours prior to the test and to fast as of 2000 hours the night prior. Subjects arrived at the GCRC at 0730 hours on the day of testing, where they remained in a supine position with their upper bodies slightly elevated. An intravenous catheter was inserted in a forearm vein where after blood samples were obtained every 10 minutes for 24 hours. A total of 488 ml (33 tablespoons) of blood was drawn over the 24-hour period. Each sample was allowed to clot at room temperature and subsequently spun in a centrifuge for 15 minutes at 2,500 rpm. Serum aliquots were transferred to storage tubes and stored in a -80°C freezer until analysis.

### **Dietary Intake during the 24-hour Analysis**

All meals for the 24-hour sampling protocol were prepared in the GCRC metabolic kitchen. Food items were measured to the nearest gram to achieve the prescribed calorie level. The diet was comprised of 55% carbohydrate, 30% fat, and 15% protein and consisted of three meals and a snack prepared for 0900 (breakfast), 1200 (lunch), 1800 (dinner), and 2100 (snack). Dinner consisted of  $502 \pm 1$  kcal, the remainder of which was distributed between breakfast ( $420 \pm 19$  kcal), lunch ( $506 \pm 25$  kcal), and the snack ( $70 \pm 3$  kcal). Subjects knew when meals were to be administered and were required to eat all/only the food provided. The caloric prescription for the 24-hour blood sampling period provided subjects with 85% of their calculated baseline energy needs to account for reductions in energy expenditure due to inactivity associated with bed rest. Meals

provided were reflective of what subjects typically consumed throughout baseline and consisted of foods like English muffins, orange juice, turkey lunchmeat sandwiches, grapes, and pork stir-fry.

### **Luteinizing Hormone**

Luteinizing hormone was measured in serum samples from the 24-hour repeated blood sampling procedure every 10 minutes from 0800 to 0800 hours using the Siemens Immulite kit (Deerfield, IL). Assay sensitivity is 0.1 mIU/mL. The intra-assay and inter-assay coefficients of variation are 5.7% and 12.3%.

### **Total Ghrelin**

Serum samples were utilized to measure total ghrelin in duplicate from the 24-hour repeated blood sampling procedure hourly from 0800 to 1000 hours, every 20 minutes from 1000 to 2000 hours and hourly from 2000 to 0800 hours using the Linco Research radioimmunoassay kit (St. Charles, MO). Assay sensitivity was 100 pg/mL. The intra-assay and inter-assay coefficients of variation for the high control were 2.7% and 16.7%, respectively. The intra-assay and inter-assay coefficients of variation for the low control were 1.2% and 14.7%, respectively. All samples from a given subject were included in the same assay.

### **24-hour Urinary Cortisol**

Twenty-four hour urinary sample aliquots were outsourced to the Pennsylvania State University Hershey campus for processing. Twenty-four hour urinary cortisol ( $\mu\text{g/dL}$ ) was analyzed by ELISA and corrected for creatinine ( $\text{mg/dL}$ ) to obtain corrected 24-hour urinary cortisol ( $\mu\text{g/g}$ ).

## **Data Analysis**

### **LH Pulse Analysis**

The time series of the 24-hour LH concentrations was analyzed for pulse frequency, peak amplitude, peak height, and 24-hour mean LH using the pulse detection algorithm Cluster (CLUSTER 8) (Veldhuis & Johnson, 1986). A 2 x 1 pulse configuration was used with a t statistic value of 2.0 for both upstroke and downstroke. Missing data was linearly interpolated between the two adjacent values. The LH variables of interest included: mean LH, LH pulse frequency, maximal peak amplitude, mean interval between LH peaks, and LH area under the curve. LH area under the curve was calculated using the computer program Cluster (Veldhuis & Johnson, 1986) and was defined as the product of the mean peak amplitude and the time of the interval.

### **Ghrelin and Cortisol Analysis**

Ghrelin data was analyzed for several descriptive parameters. Ghrelin total area under the curve (AUC) was calculated by the trapezoidal method. Twenty-four hour mean ghrelin was calculated by averaging concentrations at all time points measured in the 24-hour analysis. Peak ghrelin was designated as the highest concentration measured throughout the 24-hour analysis (typically observed in subjects prior to dinner). Change in ghrelin total AUC and change in 24-hour urinary cortisol from baseline to post-intervention were calculated by the following equation:  $(\text{post value} - \text{baseline value}) / \text{baseline value}$ .

## **Statistical Analysis**

One-way analysis of variance (ANOVA) was performed to determine statistically significant differences in demographic, fitness and body composition parameters as well as LH, ghrelin, cortisol and perceived stress variables between groups at baseline and the post-intervention time points. Paired T-tests were employed to determine significant changes in fitness, body composition, LH, ghrelin, cortisol and perceived stress parameters from baseline to post intervention in each of the subject groups. Stepwise linear regression was utilized to determine statistically significant predictors of the change in 24-hour cortisol as well as changes in LH pulsatility. All analyses were performed using IBM SPSS Statistics 19 software.

## CHAPTER 4

### RESULTS

#### Subjects

Descriptive demographics of the subjects in both the Edef group and C group at baseline are presented in Table 1. Height (cm;  $p = 0.04$ ), body weight (kg;  $p = 0.01$ ), and body fat mass (kg;  $p = 0.03$ ) were significantly higher in the Edef group ( $p < 0.05$ ), but similar in age (years;  $p = 0.42$ ), BMI ( $\text{kg}/\text{m}^2$ ;  $p = 0.35$ ), body fat percentage ( $p = 0.14$ ), fat free mass (kg;  $p = 0.18$ ), absolute (kcal/24hr;  $p = 0.50$ ) and relative (kcal/24hr/kgFFM;  $p = 0.91$ ) RMR and fitness level (ml/kg/min;  $p = 0.85$ ) when compared to the C group. In addition, no difference was observed in PSS scores between the Edef and C group at baseline ( $p = 0.92$ ).

**Table 1:** Subject Demographics at Baseline in the Control and Energy Deficit Groups

Variable	Control (n=5)		Energy Deficit (n=16)	
	Mean $\pm$ SEM	Range	Mean $\pm$ SEM	Range
Age	21.2 $\pm$ 0.7	20.0 – 24.0	20.3 $\pm$ 0.6	18.0 – 24.0
Height (cm)	159.1 $\pm$ 2.5	154.3 – 168.3	164.9 $\pm$ 1.3*	156.2 – 175.3
Weight (kg)	52.1 $\pm$ 0.9	49.1 – 54.3	58.4 $\pm$ 1.1*	51.1 – 66.5
BMI ( $\text{kg}/\text{m}^2$ )	20.6 $\pm$ 0.5	19.2 – 22.2	21.5 $\pm$ 0.5	18.4 – 24.8
Body Fat (%)	24.6 $\pm$ 2.2	19.0 – 29.5	28.3 $\pm$ 1.2	20.9 – 36.8
Body Fat (kg)	12.8 $\pm$ 1.1	9.8 – 15.6	16.6 $\pm$ 0.8*	10.7 – 22.2
Fat Free Mass (kg)	39.3 $\pm$ 1.4	36.1 – 43.6	41.8 $\pm$ 0.9	37.4 – 47.5
RMR (kcal/24hr)	1146 $\pm$ 48	1031 – 1272	1208 $\pm$ 49	919 – 1560
RMR (kcal/24hr/kgFFM)	29.4 $\pm$ 2.1	24.4 – 34.4	29.1 $\pm$ 1.3	22.3 – 39.8
VO <sub>2</sub> Max (ml/kg/min)	36.5 $\pm$ 3.1	30.9 – 41.5	37.0 $\pm$ 1.1	27.4 – 42.7
PSS	16 $\pm$ 1	12 – 18	16 $\pm$ 2	6 – 34

\* $p < 0.05$

### Changes in Descriptive Parameters from Baseline to Post-Intervention

Table 2 demonstrates changes in body composition, resting metabolism, fitness and perceived stress parameters from baseline to post-intervention. Body weight, BMI, body fat percentage and body fat mass significantly decreased from baseline to post intervention ( $p < 0.001$ ). Demonstrating the effect of the exercise intervention,  $VO_{2max}$  increased significantly ( $p = 0.001$ ) whereas fat free mass ( $p = 0.63$ ) remained constant. As expected, body composition and fitness remained unchanged in the C group from baseline to post. Additionally, no change was observed in PSS from baseline to post-intervention in either group ( $p > 0.05$ ).

TABLE 2: Change in Body Composition, Fitness and Perceived Stress from Baseline to Post-Intervention in the Control and Energy Deficit Groups

Variable	Control (n=5)			Energy Deficit (n=16)		
	Baseline (Mean $\pm$ SEM)	Post (Mean $\pm$ SEM)	p-value	Baseline (Mean $\pm$ SEM)	Post (Mean $\pm$ SEM)	p-value
Weight (kg)	52.1 $\pm$ 0.9	50.9 $\pm$ 1.1	0.146	58.4 $\pm$ 1.1	55.3 $\pm$ 1.2	<0.001*
BMI (kg/m <sup>2</sup> )	20.6 $\pm$ 0.5	20.1 $\pm$ 0.3	0.151	21.5 $\pm$ 0.5	20.4 $\pm$ 0.5	<0.001*
Body Fat (%)	24.6 $\pm$ 2.2	23.2 $\pm$ 1.8	0.162	28.3 $\pm$ 1.2	24.6 $\pm$ 1.2	<0.001*
Body Fat (kg)	12.8 $\pm$ 1.1	11.9 $\pm$ 0.9	0.174	16.6 $\pm$ 0.8	13.7 $\pm$ 0.9	<0.001*
Fat Free Mass (kg)	39.3 $\pm$ 1.4	39.0 $\pm$ 1.3	0.508	41.8 $\pm$ 0.9	41.6 $\pm$ 0.8	0.626
$VO_2$ Max (ml/kg/min)	36.2 $\pm$ 5.3	38.0 $\pm$ 1.3	0.731	37.1 $\pm$ 1.1	43.0 $\pm$ 1.5	0.001*
PSS	16 $\pm$ 1	21 $\pm$ 4	0.171	16 $\pm$ 2	18 $\pm$ 2	0.402

\* $p < 0.05$

### LH Concentration Changes Baseline to Post-Intervention

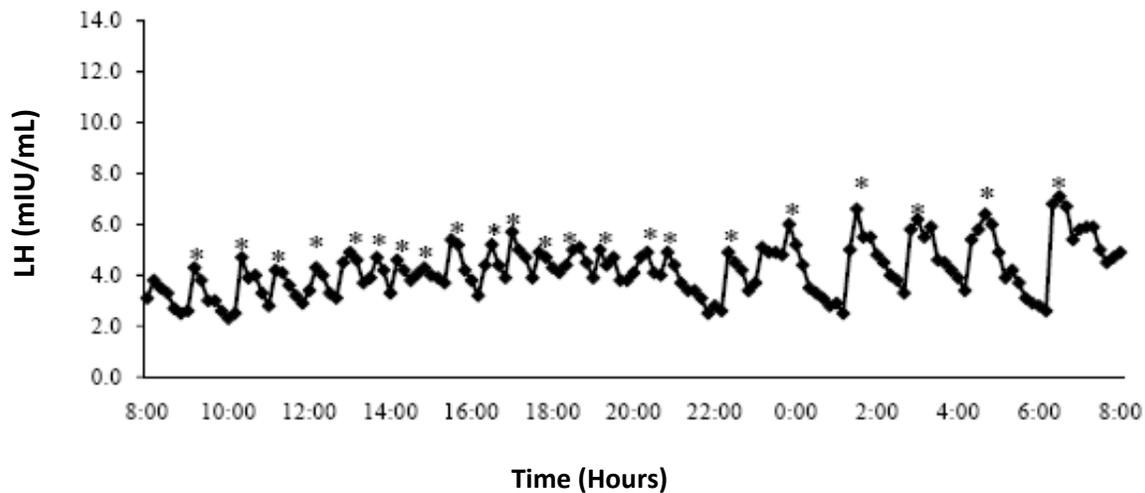
Table 3 exhibits the changes from baseline to post-intervention in LH pulse frequency, peak amplitude and peak interval for both groups. LH pulse frequency significantly decreased in the Edef group from baseline to post-intervention ( $p = 0.047$ ). No change was observed in either peak amplitude or peak interval in the Edef group ( $p > 0.05$ ). As expected, no changes were observed in LH pulse frequency, peak amplitude or peak interval in the C group ( $p > 0.05$ ).

Figure 1 depicts LH pulse frequency over one 24-hour period at baseline (Figure 1A) and post-intervention (Figure 1B) in one subject in the Edef group. These graphs demonstrate a typical example of the suppression of LH pulse frequency observed from baseline to post-intervention in response to the 3-month intervention.

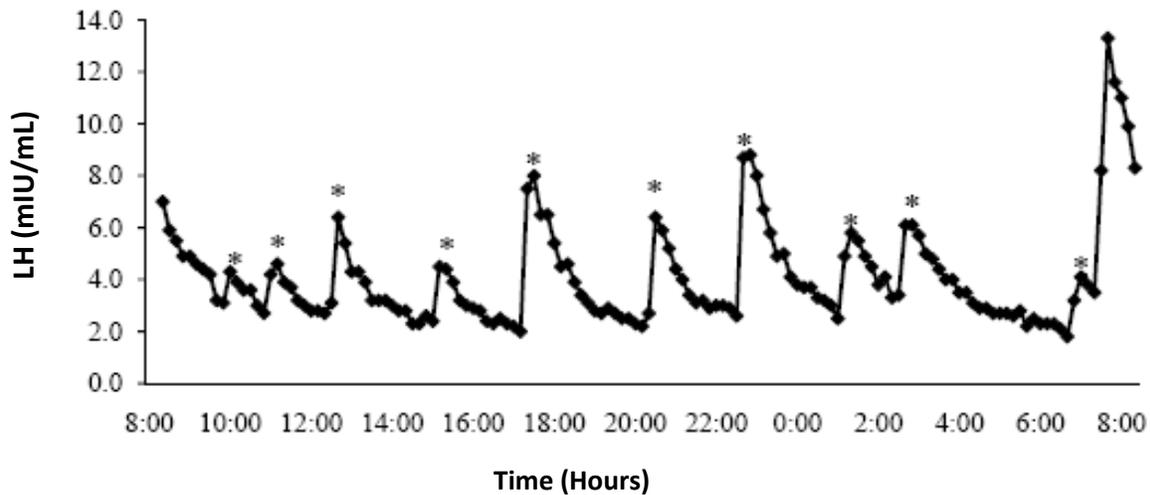
**TABLE 3: Change in LH, Ghrelin and Cortisol from Baseline to Post-Intervention in the Control and Energy Deficit Groups**

Variable	Control (n=5)			Energy Deficit (n=16)		
	Baseline (Mean $\pm$ SEM)	Post (Mean $\pm$ SEM)	p-value	Baseline (Mean $\pm$ SEM)	Post (Mean $\pm$ SEM)	p-value
<b>LH</b>						
LH Pulse Frequency (pulse/hr)	0.76 $\pm$ 0.11	0.75 $\pm$ 0.12	0.928	0.81 $\pm$ 0.06	0.63 $\pm$ 0.08	0.047*
Peak Amplitude (mIU/ml)	10.3 $\pm$ 0.7	10.4 $\pm$ 0.947	0.607	9.6 $\pm$ 0.6	10.4 $\pm$ 1.0	0.248
Peak Interval (min/peak)	85.7 $\pm$ 12.3	86.3 $\pm$ 10.7	0.967	85.7 $\pm$ 13.1	108.7 $\pm$ 21.5	0.445
<b>Cortisol</b>						
24hr Urinary Cortisol ( $\mu$ g/g)	44.7 $\pm$ 9.3	56.1 $\pm$ 13.4	0.270	40.7 $\pm$ 3.4	57.3 $\pm$ 5.9	0.018*
<b>Ghrelin</b>						
Total AUC (pg/ml*24hr)	37663 $\pm$ 2885	37952 $\pm$ 3752	0.847	28903 $\pm$ 2739	34799 $\pm$ 3382	0.002*
24hr Mean (pg/ml)	1616.0 $\pm$ 122.2	1634.2 $\pm$ 178.3	0.822	1216.9 $\pm$ 126.3	1457.8 $\pm$ 150.3	0.002*
Peak (pg/ml)	2053.0 $\pm$ 165.2	2174.5 $\pm$ 207.8	0.160	1602.4 $\pm$ 153.2	2007.2 $\pm$ 214.7	0.003*

\* $p < 0.05$



**Figure 1A:** LH Pulse Frequency over 24 hours in One Edef Subject at Baseline. \* indicates significant pulses in LH.



**Figure 1B:** LH Pulse Frequency over 24 hours in One Edef Subject at Post-Intervention. \* indicates significant pulses in LH.

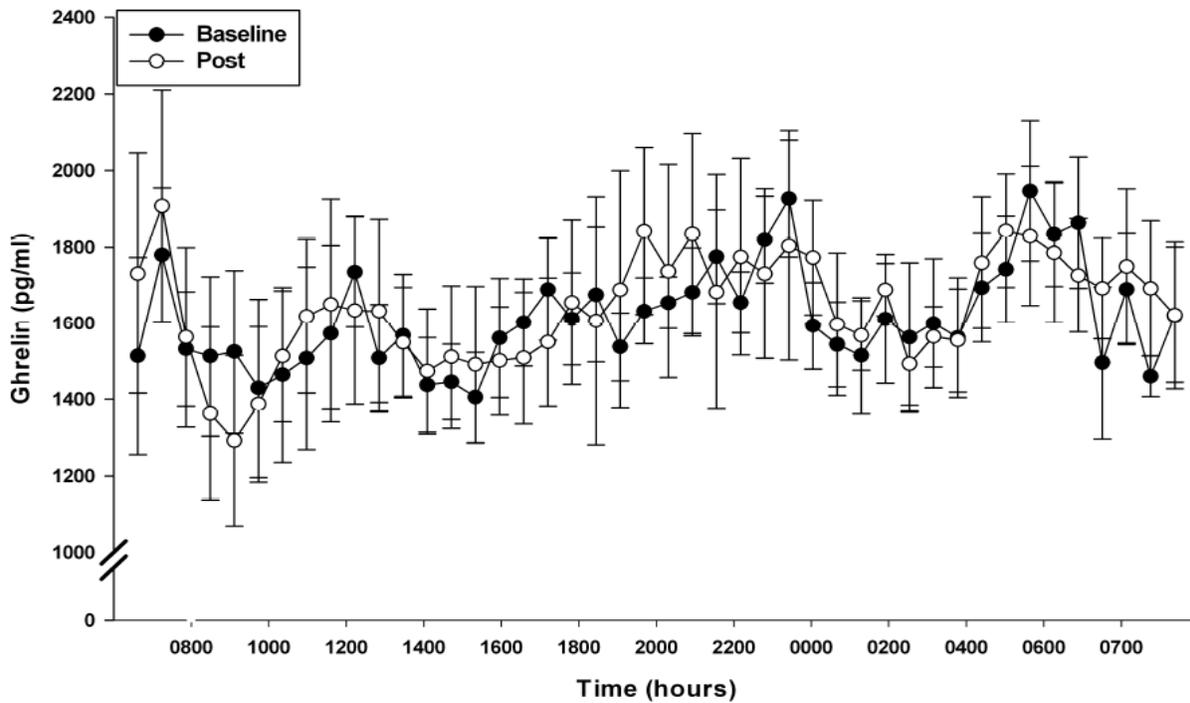
### **Cortisol Concentration Changes Baseline to Post-Intervention**

The changes from baseline to post-intervention in 24-hour urinary cortisol for the Edef and C groups are also exemplified in Table 3. Twenty-four hour cortisol increased significantly from baseline to post intervention in response to weight loss in the Edef group ( $p = 0.018$ ). Additionally, no change was observed in the C group ( $p > 0.05$ ).

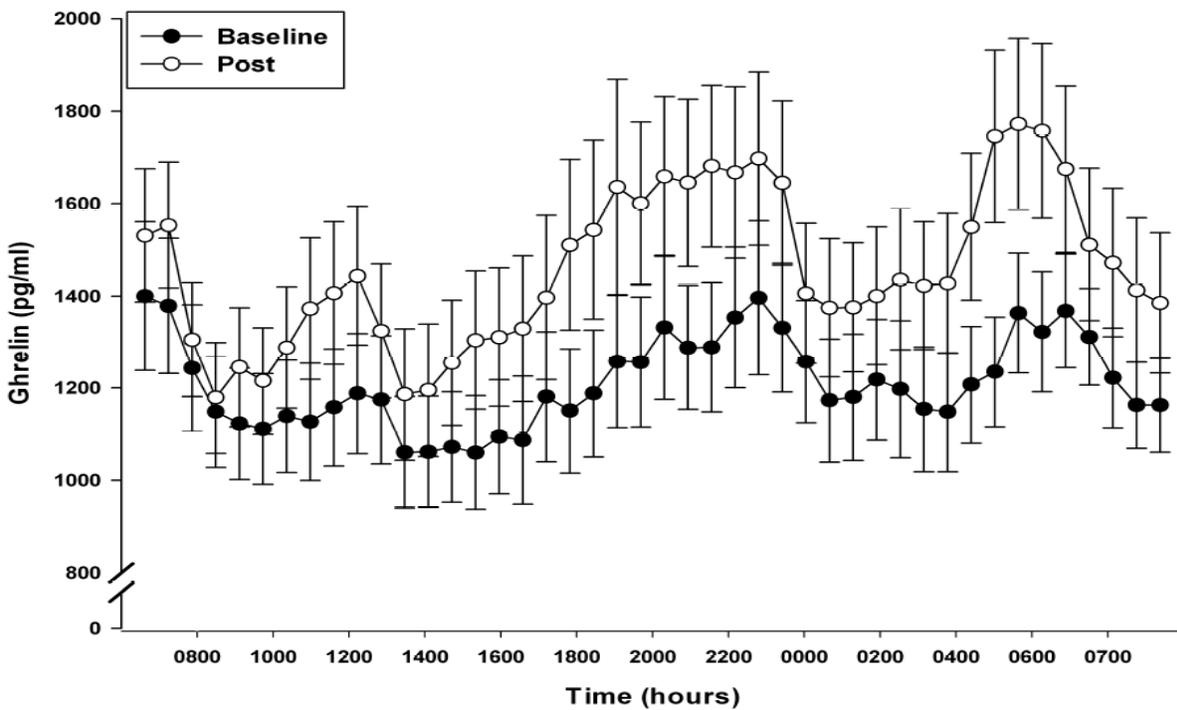
### **Ghrelin Concentration Changes Baseline to Post-Intervention**

Table 3 also displays the changes in ghrelin total AUC, 24-hour mean ghrelin and peak ghrelin for both groups from baseline to post-intervention. Total AUC ( $p = 0.002$ ), 24-hour mean ( $p = 0.002$ ) and peak ( $p = 0.003$ ) all significantly increased in response to the intervention in the Edef group. No changes were observed in any of these same measures in the C group ( $p > 0.05$ ).

Figures 2 illustrates the 24-hour profile of total circulating ghrelin from baseline to post-intervention in the C group (Figure 2A) and Edef group (Figure 2B). This further displays the elevation in ghrelin concentrations observed in the Edef group in response to the intervention, which is not detected in the C group.



**Figure 2A:** Circulating Concentrations of 24-hour Ghrelin in the Control Group at Baseline (●) and Post-Intervention (○)



**Figure 2B:** Circulating Concentrations of 24-hour Ghrelin in the Edef Group at Baseline (●) and Post-Intervention (○)

## **Relationships of LH, Cortisol and Ghrelin**

Table 4 indicates the results of the stepwise linear regression utilized to determine significant predictors of the change in 24-hour cortisol as well as the change in LH pulse frequency from baseline to post-intervention. Our lab has previously demonstrated the association between ghrelin and LH pulse frequency (unpublished data) to which we have attempted to incorporate changes in 24-hour cortisol within the regression analysis to determine if cortisol modulates this association.

We hypothesized that the change in 24-hour cortisol would significantly predict changes observed in LH pulse frequency such that elevations in 24-hour cortisol would be associated with suppression of LH pulse frequency; however, we were unable to demonstrate this association upon the addition of the change in 24-hour cortisol to the regression analysis. The change in 24-hour mean ghrelin was the only significant predictor of the changes in LH pulsatility demonstrating that increases in 24-hour mean ghrelin were associated with decreases in LH pulse frequency from baseline to post intervention ( $p = 0.01$ ).

We were unable to demonstrate an association between 24-hour cortisol concentrations and LH pulse frequency; however, we were able to demonstrate an association between ghrelin and 24-hour cortisol. Significant predictors of the change in 24-hour cortisol were the change in body weight ( $p = 0.03$ ) and the change in ghrelin AUC ( $p < 0.001$ ) indicating that weight loss as well as increases in ghrelin are associated with increases in 24-hour cortisol from baseline to post-intervention.

Table 4: Predictors of the Change in 24-hour Cortisol and Change in LH Pulsatility from Baseline to Post-Intervention

Outcome Variable	Predictor Variable	Coefficient	p-value
Change in LH Pulsatility	Constant	0.02	0.84
	Change in Body Weight (kg)	0.15	0.51
	Change in BMI (kg/m <sup>2</sup> )	0.15	0.51
	Change in Body Fat (kg)	0.06	0.78
	Change in Fat Free Mass (kg)	0.05	0.84
	Change in Ghrelin AUC (pg/mlx24h)	1.28	0.21
	Change in Ghrelin 24-hour Mean (pg/ml)	- 0.96	0.01*
	Change in Ghrelin Peak (pg/ml)	0.47	0.16
	Change in 24-Hour Cortisol (µg/g)	0.14	0.57
Change in 24-Hour Cortisol	Constant	0.33	0.08
	Change in Body Weight (kg)	7.58	0.03*
	Change in Ghrelin AUC (pg/mlx24h)	2.83	<0.001*
	Change in Ghrelin 24-hour Mean (pg/ml)	- 1.55	0.10
	Change in Ghrelin Peak (pg/ml)	0.13	0.66
	Change in Perceived Stress Score	0.04	0.10

\*p &lt; 0.05

## CHAPTER 5

### DISCUSSION

In exercising women with EAMD, circulating concentrations of ghrelin have been shown to be elevated, whereas LH pulse frequency, a surrogate marker of menstrual function, has been shown to be suppressed. The association between ghrelin and LH has been previously demonstrated in both human (Kluge, Schüssler, Uhr, Yassouridis, & Steiger, 2007) and animal models (Vulliémoz, et al., 2004). This association may suggest a role for ghrelin in the suppression of LH pulse frequency and thus menstrual disturbances in exercising women. Additionally, the association between ghrelin and cortisol has been demonstrated in animal models such that elevated concentrations in ghrelin elicited a stimulation of cortisol release. However, this association has yet to be exhibited in humans. Here we demonstrated that, in response to diet and exercise induced weight loss, ghrelin and 24-hour urinary cortisol increased and LH pulse frequency decreased. Furthermore, we demonstrated that increases in circulating concentrations of ghrelin were associated with increases in 24-hour cortisol in response to an energy deficit and subsequent weight loss. Though it has been suggested that cortisol may be involved in the modulation of LH pulsatility (Padmanabhan, Keech, & Convey, 1983), we were unable to corroborate this association in our population.

The pulsatile release of LH from the pituitary gland is critical to estrogen production in the ovaries and overall healthy reproductive function. Though important for fertility, this bodily system is susceptible to suppression by an unknown mechanism in times of energy deficiency as there are other systems necessary to sustaining life, for example brain and cellular function, that require this energy to be conserved (Wade, Schneider, & Li, 1996).

Recent research has observed a high incidence of reproductive suppression, such as FHA and other, more subtle menstrual disturbances in exercising women (De Souza, et al., 2007). We have demonstrated that diet- and exercise-induced weight loss in response to an energy deficit elicited a suppression of LH pulse frequency in women who were sedentary and weight stable at baseline. This corroborates the hypothesis that menstrual disturbances occur in response to an energy deficit as opposed to the stress of exercise as has been previously demonstrated in Rhesus monkeys (Williams, et al., 1995).

It has been hypothesized that subsequent to an energy deficit, metabolic changes occur that signal to GnRH neurons to suppress GnRH pulsatility. The suppression of GnRH pulses leads to the suppression of LH pulsatility and subsequently, the entire reproductive axis (Wade & Jones, 2004). This suppression of both GnRH and LH pulsatility has been demonstrated in AN, the most severe form of energy deficiency (Katz, Boyar, Roffwarg, Hellman, & Weiner, 1978).

Elevated concentrations of cortisol have been hypothesized to be one possible factor involved in the inhibition of GnRH as well as LH pulsatility. Elevations in CRH (Chen, O'Byrne, Chiappini, Hotchkiss, & Knobil, 1992) and the presence of glucocorticoid receptors on GnRH neurons in the hypothalamus (Chandran, et al., 1994) may implicate that cortisol is involved in the modulation of GnRH pulsatility. Additionally, one study in a bovine model observed that in vitro cortisol administration to pituitary cells inhibited LH release (Padmanabhan, Keech, & Convey, 1983). Moreover, cortisol concentrations have been shown to be elevated in energy deficient women suffering from AN (Misra, et al., 2004). In the current study, we hypothesized that cortisol would be an intermediary factor in the association between ghrelin and LH. We exhibited increases in concentrations of 24-hour

urinary cortisol in response to an energy deficit and weight loss; however, were unable to demonstrate an association between cortisol and LH pulsatility. Arimura et al (2010) demonstrated that baseline serum cortisol was the only significant predictor of the lack of resumption of menses in women with AN and thus, cortisol may be involved in the suppression of reproductive function by mechanisms other than the direct suppression of LH pulse frequency.

In addition to cortisol, elevations in ghrelin have also been reported in women with EAMD (Scheid, De Souza, Leidy, & Williams, 2011) as well as AN (Otto, et al., 2001). We hypothesized that elevated circulating concentrations of ghrelin observed in response to weight loss would be associated with increases in 24-hour cortisol. Recently, a possible role for ghrelin in the suppression of LH pulsatility has been demonstrated (Kluge, Schüssler, Uhr, Yassouridis, & Steiger, 2007). Additionally, ghrelin infusion has also been shown to elicit a stimulation of the secretion of cortisol (Vulliémoz, et al., 2004). However, dissimilar to cortisol, the growth hormone secretagogue receptor (GHS-R), the receptor to which ghrelin binds, has not been discovered on GnRH neurons (Guan, et al., 1997). The culmination of these findings suggests an interaction may exist among these three hormones in the suppression of the menstrual cycle in response to an energy deficit. In the present study, we demonstrated an association between ghrelin and cortisol such that elevated 24-hour mean ghrelin was associated with increases in 24-hour urinary cortisol, but were unable to connect any association between cortisol and LH. To our knowledge, this is the first account of the association between cortisol and ghrelin in normal weight, premenopausal women in response to an energy deficit.

Strengths of our study are several-fold. Dietary intake was prescribed and provided to subjects to accurately quantify food consumed. Additionally, energy expenditure was prescribed, supervised and quantified by lab technicians using a polar heart rate monitor to quantify energy expended per bout. Lastly, menstrual cycle characterization was completed by collection of urinary hormone samples which is unique to our lab and is the most accurate account of menstrual cycle disturbances as most studies utilize self-reported menstrual history. One limitation to the study is that cortisol was only quantified using 24-hour urinary measurements and not serum cortisol. Also, the limited size of the sample in this study reduces statistical power of our results. We may have been able to demonstrate the association between cortisol and LH pulse frequency had we had a larger subject population. Future studies in animal models could create a ghrelin knock-out model and infuse cortisol to determine its true association to LH.

In conclusion, ghrelin may be involved in the modulation of cortisol as well as LH pulsatility; however, we have been unable to demonstrate the relationship between cortisol and LH and thus, ghrelin may be acting through other hypothalamic neuroendocrine factors such as neuropeptide Y (NPY), kiss-peptin or proopiomelanocortin (POMC) to suppress LH pulsatility. Cortisol may be also be involved in the suppression of the HPO axis, but is likely not an intermediary in the association between ghrelin and LH.

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## ACADEMIC VITA

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### Present Address

456 E. Beaver Avenue Apt. 205  
State College, PA 16801

### Permanent Address

221 Leaf Lane  
Hollidaysburg, PA 16648

### EDUCATION

**The Pennsylvania State University**, University Park, PA  
Bachelor of Science Degree in Biobehavioral Health, Spring 2012  
Minors in Biology, Neuroscience and Psychology

Honors in Kinesiology, Schreyer Honors College, Spring 2012

### THESIS

***The Association between Weight Loss Induced Suppression of LH Pulsatility and Metabolic Hormones***

Supervised by Dr. Nancy I. Williams

### RELATED WORK EXPERIENCE

**The Women's Health and Exercise Laboratory**, Noll Laboratory, University Park, PA  
*Undergraduate Honors Thesis Student and Research Assistant* **January 2011-present**

- Acquired a depth of understanding and affinity about the endocrine system and the body's response to hormones
- Aided in the preparation of biological samples for analysis through aliquotting urine from the sample cups, measuring specific gravity and ensuring all necessary data is collected and filed properly
- Collaborated on successful scientific writings

**The Penn State Biology Department**, Mueller Laboratory, University Park, PA  
*Teaching Assistant for Biology 110* **June 2011-December 2011**

- Taught a class of approximately 24 students during the Summer 2011 and Fall 2011 semesters
- Wrote quizzes on lab topics and graded them along with homework and writing assignments
- Mastered concepts in basic biology and laboratory techniques

**The Mount Nittany Medical Center**, State College, PA  
*Volunteer* **May 2011-August 2011**

- Aided nurses and physicians with preparing IV bags and shadowing several surgeries
- Sanitized and made new hospital beds for incoming patients, stocked the Surgery Center recovery room and changed the laundry
- Observed the interactions among patients, families and all hospital personnel demonstrating the importance of good patient-physician relationships

**The Mind-Body Cardiovascular Psychophysiology Laboratory, University Park, PA**

*Undergraduate Research Assistant*

**August 2009-December 2010**

- Performed basic research activities such as interacting directly with participants, editing research scripts and protocols, and manipulating raw data in statistic tables to yield readable results
- Completed various lab assignments, literature searches, and research database formulations
- Observed the beginnings of two new research studies and how they progress through trial and error periods

**LEADERSHIP AND EXTRACURRICULAR ACTIVITIES**

**New Member Orientation and Training Coordinator 2011-2012, External Relations Advisor**

**2010-2011** Penn State Lion Scouts

- Led the recruitment and selection process of new members and planned their training and orientation activities
- Coordinated the overnight retreat for both new and current members
- Conducted development activities with local business owners for our organization

**Rules and Regulations Committee Penn State Dance Marathon 2011-2012**

- Enforced security protocols for the Bryce Jordan Center to enforce during THON weekend in order to keep all attendees safe
- Helped raise \$10.68 million for the Four Diamonds Fund at Penn State Hershey Medical Center aiding both financial and emotional support to families and patients battling pediatric cancer

**Tour Guide Penn State Lion Scouts 2008-present**

- Directed and educated prospective students and families around the Penn State University Park campus
- Communicated with prospective students in person and via internet to answer questions and aid in their college decision process
- Attended several events promoting the morale of the organization and demonstrating the pride that is Penn State University

**AWARDS**

**Pennsylvania State University Class of 1922 Memorial Scholarship**

July 2011

**Dean's List:** Fall 2008, Spring 2009, Fall 2009, Spring 2010, Fall 2010, Spring 2011, Fall 2011