THE ROLE OF PEPTIDE YY IN ENERGY HOMEOSTASIS

A Dissertation in

Physiology

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2013
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ABSTRACT

The primary purpose of this dissertation is to understand the physiological role of peptide YY (PYY) in response to both acute and chronic changes in energy balance and body weight in women. The specific goals of this dissertation are to characterize the diurnal rhythm of PYY with regard to acute dietary energy intake and explore its role in energy balance, to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation both prior and subsequent to diet- and exercise-induced weight loss, and to determine whether low-energy dense diets might facilitate weight loss through actions on circulating concentrations of ghrelin and PYY. Study 1 was designed to characterize features of the diurnal rhythm of PYY and to explore the role of PYY in energy balance in normal weight premenopausal women. We demonstrated that PYY displays a meal-driven diurnal rhythm and is correlated to resting metabolic rate, a major contributor to energy expenditure. Thus, PYY varies in accordance with energy content and RMR, supporting a role for PYY in energy balance modulation. Study 2 was designed to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation during a period of weight stability in non-exercising, normal weight premenopausal women. We demonstrated that circulating concentrations of PYY were inversely associated with ghrelin over an entire 24 hour period. We concluded that these data provide evidence that PYY may contribute to the modulation of the secretion of ghrelin in normal weight, premenopausal women over a 24 hour period which supports similar inferences from experimental studies in animals and humans. The purpose of Study 3 was designed to determine if diet- and exercise-induced
weight loss would alter the 24-hour profile of total circulating PYY and to determine if the association between 24-hour circulating concentrations of PYY and ghrelin observed during a period of weight stability (study 2) will be altered subsequent to a period of diet- and exercise-induced weight loss in normal weight, premenopausal women. We demonstrated that no change occurred in the 24-hour profile of total circulating PYY subsequent to diet- and exercise-induced weight loss and that the association observed at baseline between ghrelin and PYY (Study 2) was weakened subsequent to diet- and exercise-induced weight loss where circulating concentrations of ghrelin increased despite administration of exactly the same calorie and macronutrient content of meals during the 24-hour analyses. We also created a variable to illustrate an index of hormonal exposure in an attempt to demonstrate the potential predominance of ghrelin over PYY in the circulation in response to weight loss. To accomplish this, we calculated the ghrelin/PYY ratio over 24 hours at baseline and the post intervention time points. The lunch ghrelin/PYY mean was significantly higher at post intervention and trends toward significant increases were observed in the dinner and nocturnal ghrelin/PYY means demonstrating that the time frame during which uncoupling of the ghrelin and PYY profiles occurred was mainly throughout the lunch to dinner time period. This uncoupling may represent periods during which there is a predominance of ghrelin over PYY in the circulation subsequent to weight loss which may promote weight regain. The purpose of Study 4 was to determine whether low-energy dense diets might facilitate weight loss through actions on circulating concentrations of ghrelin and PYY, independent of the influence of psychosocial measures of dietary restraint, disinhibition, and tendency toward hunger in overweight and obese women. We demonstrated that reductions in
dietary energy density may promote weight maintenance after a period of weight loss by opposing increases in ghrelin in response to an energy deficit that can lead to weight regain, as well as by promoting increases in circulating concentrations of the satiety hormone PYY. Overall, ghrelin and PYY are likely important factors involved in the response to, as well as the modulation of body weight regulation in women. As well, ghrelin and PYY may modulate on another during a period of weight stability, but that association may be altered in a state of energy imbalance where the 24-hour profile of ghrelin, but not PYY is elevated. Lastly, low energy dense diets may impact circulating concentrations of ghrelin and PYY in such a manner as to promote satiety and prevent weight re-gain subsequent to a period of weight loss.
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Acknowledgments

This dissertation would not have been possible without the support and guidance of my mentor, Dr. Nancy Williams. Thank you for challenging me throughout my doctoral work and facilitating the progress of my career as a future researcher. It is because of you that I have developed my ability to think independently and critically. It was a privilege and honor working with you.

I would like to thank Dr. Mary Jane De Souza for offering mentorship throughout my doctoral studies at the Pennsylvania State University. Your support and encouragement have been greatly appreciated along with the opportunities you have offered and training you have provided regarding data analysis and scientific writing.

I would like to thank Dr. Barbara Rolls for affording me the opportunity to collaborate with her laboratory on my fourth dissertation manuscript and for serving on my dissertation committee. As well, I thank Dr. Michael Green for serving on my dissertation committee. I am grateful for your feedback and suggestions in helping to improve this dissertation. It was a pleasure working with you.

I am grateful to my colleagues in the Women's Health and Exercise Lab, the nurses and staff of the General Clinical Research Center, the Intercollege Graduate Degree Program in Physiology and the Departments of Kinesiology and Biology for your assistance, support, and encouragement throughout my doctoral studies and in helping to make this dissertation possible.

Finally, I would like to thank my family, friends, and most importantly, my husband, Ryan Hill, for his love, support, and patience throughout my graduate career. This dissertation would not have been possible without you.
Chapter 1
Introduction

Peptide YY (PYY) is secreted from L-cells in the gut where it slows digestion, acting as an “ileal brake” to increase absorption of nutrients [1]. PYY₁₋₃₆, cleaved to PYY₃₋₃₆ via dipeptidyl peptidase, is secreted in proportion to energy and macronutrient content, where high-fat or high-protein meals stimulate greater PYY secretion than high-carbohydrate meals [2-4]. PYY reaches peak concentrations 1-2 hours postprandially and aids in meal cessation [2] and has consequently been identified as a satiety hormone.

Fasting and postprandial PYY concentrations are suppressed in obese individuals and elevated in energy deficient women [5-8]. To date much of the work in humans has been observations of single-meal responses in pathophysiological conditions [4, 6-9] where normal physiological responses have been perturbed.

The diurnal rhythms of hormones like ghrelin and leptin have been characterized in terms of responses to meal timing [10-12], energy content of meals [13], and the impact of energy deficiency [13, 14]. The diurnal rhythm of PYY has yet to be fully characterized under normal physiological conditions, or in relation to meal timing and meal energy or macronutrient content. Studies of single-meal responses do not address whether PYY responses change across the day due to time of day or as nutrient intake accumulates.

As well, studies have also suggested a potential role for PYY in the modulation of energy expenditure [15, 16]. Guo et al. [17] found a negative correlation between fasting PYY and resting metabolic rate as well as respiratory quotient and postprandial peak PYY. Additionally, Sloth et al. [15, 18] peripherally infused PYY and showed increases
in energy expenditure (kJ/day) as well as fat oxidation (lowered respiratory quotient) in lean and obese men.

Cumulatively, studies in individuals without chronic disease may aid in our understanding of the role of PYY in the etiology of disease. As well, the role of PYY in energy balance, particularly with regard to energy expenditure, is unclear as evidenced by several disparities in the current literature which may be the result of studying varying populations [5, 7, 9, 17]. To address these gaps in the literature, we conducted a cross-sectional analysis in non-exercising, normal weight premenopausal women designed to characterize features of the diurnal rhythm of PYY and to explore the role of PYY in energy balance in normal weight, premenopausal women. From this first study, we hoped to gain understanding regarding the modulation of PYY secretion across the day in normal weight individuals to provide a valuable reference to which results from studies involving abnormal states of energy balance can be compared.

Studies using animal models [19] and humans [13, 20] support the roles of ghrelin and PYY in the modulation of energy intake. Whereas PYY is one of several satiety hormones, ghrelin is the only peripherally secreted hunger hormone that has been identified to date. It is hypothesized that PYY and ghrelin modulate energy balance through integration within the hypothalamic arcuate nucleus [21]. Evidence to support this was provided by Riedeger et al. [22] in male Wistar rats where intra-arcuate injection of PYY inhibited the activation of ghrelin-sensitive neurons. However, PYY and ghrelin are secreted from cells along the gastrointestinal (GI) tract where secretion is largely modulated by caloric as well as macronutrient content of meals [13, 20]. As well, it has been demonstrated that ghrelin and PYY oppose the actions of one another on the GI
tract: PYY slows GI motility and suppresses gastric acid secretion [23] and ghrelin stimulates those same actions [24]. To that end, one study demonstrated that intravenous infusion of ghrelin attenuated the effects of PYY infusion on gastric acid secretion and GI motility [25]. Therefore, focusing on hypothalamic regulation of these hormones may limit our understanding of their reciprocal actions in the peripheral circulation.

Although PYY and ghrelin may reciprocally inhibit the actions of one another on the GI tract, the mechanism through which PYY and ghrelin may modulate the actual secretory profiles of one another in the periphery is unknown [26]. Evidence to support a regulatory association between PYY and ghrelin was demonstrated when infusion of PYY in the fasted state of obese and lean individuals suppressed pre- and postprandial circulating concentrations of ghrelin [8]. In light of this, it is attractive to hypothesize that ghrelin and PYY may be directly involved in modulating the secretion of one another in a negative feedback manner in the periphery. However, studies that have demonstrated an association between PYY and ghrelin in the circulation have mainly demonstrated this association in response to a single meal [8]. And no study has examined whether an inverse association between PYY and ghrelin exists in the peripheral circulation of healthy, normal weight humans over an entire 24 hour period.

Additionally, we previously reported that the 24-hour profile of ghrelin is elevated subsequent to diet- and exercise-induced weight loss in normal weight premenopausal women [12]. However, no study has determined if the association between 24-hour profiles of ghrelin and PYY demonstrated during a period of weight stability remains subsequent to diet- and exercise-induced weight loss.
To address these gaps in the literature, we conducted a cross-sectional analysis in non-exercising, normal weight premenopausal women designed to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation during a period of weight stability. As well, we conducted a 3-month controlled feeding and exercise intervention in these same participants to determine if diet- and exercise-induced weight loss would alter the 24-hour profile of total circulating PYY or its association to 24-hour ghrelin. From these second and third studies, we hoped to gain insight as to whether PYY would be inversely associated with ghrelin over 24 hours both before and after weight loss to potentially provide insight as to how these hormones may be involved in the modulation of one another in a feedback mechanistic manner. Information from the outcomes of these studies may provide insight into specific mechanisms of actions of these hormones and how aberrant profiles of hormones may predispose individuals to pathophysiological conditions such as obesity. As well, these data may provide a better understanding of hormone interactions that could be utilized in drug therapies to prevent or treat disease. Additionally, we hoped to gain an understanding of how changes in body weight impact the 24-hour profile of PYY and its association to 24-hour ghrelin in the hope that determining how the association between PYY and ghrelin changes in response to weight loss may impact chronic body weight regulation.

Dietary energy density (ED; kcal/g) can be reduced by decreasing the proportion of fat or by increasing the water content of foods [27, 28] facilitating the reduction of energy intake while maintaining the volume of food eaten. Decreasing dietary ED has been shown to be useful in long-term weight loss [29-33]; however, the underlying
physiological mechanisms remain to be elucidated, particularly with regard to key gut hormones involved in food intake regulation such as ghrelin and PYY.

Circulating ghrelin increases with weight loss in normal weight women [12, 34], whereas concentrations are suppressed and normalized (increased) with weight loss in obese individuals [35]. PYY concentrations are suppressed and increased energy intake is needed to stimulate equivalent PYY secretion in obese individuals in comparison to normal weight counterparts [7]. With weight loss, circulating PYY does not necessarily return to concentrations observed in normal weight counterparts. Some studies have demonstrated increases [9, 36] whereas others have observed decreases [37-39] or no change [40] in PYY in response to weight loss. Additionally, ghrelin and PYY may be related to psychosocial measures of eating behaviors such as dietary restraint (tendency to consciously restrict food intake to control body weight) [41, 42], disinhibition (loss of control over eating in response to emotional or social cues) [43, 44], and tendency toward hunger [45]. These studies have begun to relate behavioral to physiological measures; however, interactions between these factors have not been determined. It is attractive to posit that altered physiological states, such as suppressed concentrations of PYY, may be modulating behavioral changes, such as increases in dietary restraint, to offset biological abnormalities that may influence meal-related changes in appetite.

To address this gap in the literature, we conducted a secondary analysis from a previous year-long clinical trial in which obese women received instruction in reducing dietary ED to promote weight loss [30]. Blood samples collected during the trial provided an opportunity to investigate how physiological mechanisms such as changes in gut hormones, namely PYY and ghrelin, in response to modifications in dietary ED may
promote weight loss and weight maintenance. Additionally, we sought to determine if the impact of ED on PYY and ghrelin remains significant when accounting for changes in psychosocial variables. Outcomes from this fourth study will be the first to examine the effect of low-ED diets on alterations in GI hormone profiles during weight loss and weight loss maintenance. We hope to gain insight into the potential protective effect that low-ED diets might provide against weight regain subsequent to a period of weight loss. Outcomes from this study may aid in creating effective dietary prescriptions in individuals seeking weight loss and chronic weight loss maintenance.

When taken together, the overall goal of these four studies is to help increase our understanding of the circulating profiles of PYY and ghrelin and their association with each other over 24-hours during a period of weight stability as well as subsequent to weight loss. Additionally, these studies will help increase the understanding of the underlying mechanism through which low-ED diets may facilitate long-term weight loss. Outcomes from these studies may provide insight into the hormonal modulation of energy balance in normal weight women in the hope of providing a better understanding of hormone interactions that could be utilized in dietary or perhaps, drug therapies to prevent, manage or treat disease.
Chapter 2
Review of Literature

Part 1


Abstract

Chronic energy deficiency may suppress reproduction to preserve energy for life-sustaining physiological functions; however, the mechanism is unclear. It is believed that hormones such as ghrelin and leptin signal a state of low energy availability (EA) to the hypothalamus leading to the suppression of gonadotropin-releasing hormone (GnRH) secretion. GnRH suppression inhibits the pulsatile release of luteinizing hormone resulting in inhibition of folliculogenesis and ovulation. Exercising women may be at an increased risk for the Female Athlete Triad, a syndrome consisting of three interrelated clinical conditions: low EA, menstrual disturbances and low bone mass. The Triad’s etiology has been attributed to chronic energy deficiency induced by inadequate energy intake to compensate for exercise energy expenditure. Unhealthy dietary strategies to maintain low EA include clinically diagnosed eating disorders of which amenorrhea is a diagnostic criterion, disordered eating which has also been linked to menstrual dysfunction and includes dieting, food avoidance, and vegetarianism, and inadvertent under-eating. Aberrant eating attitudes may lead to/exacerbate disordered eating and have also been linked to menstrual disturbances. Exercise may acutely suppress appetite, elicit an anorexigenic gastrointestinal hormone profile and alter food reward responses;
however, more studies are needed to address whether inadvertent under-eating contributes to menstrual dysfunction.

**Introduction**

The hypothalamic-pituitary-ovarian (HPO) axis is sensitive to fluctuations in energy balance. In conditions of energy deficiency, reproductive function may be suppressed to redirect energy away from the energy-costly process of reproduction to life-sustaining functions such as cellular maintenance and thermoregulation. Exercising women commonly experience low energy availability by decreasing dietary energy intake, increasing energy expended through exercise, or both. Chronic energy deficiency and consequent suppression of reproductive function may manifest as the Female Athlete Triad, a syndrome of interrelated conditions that exist along a spectrum of severity ranging from optimal energy balance, regular, ovulatory menstrual cycles and healthy bone mineral density to low energy availability with or without disordered eating, amenorrhea and osteoporosis [46]. Exercising women may exhibit chronically low energy intake by way of clinically diagnosed eating disorders such as anorexia nervosa (AN) or bulimia nervosa (BN); however, there is an even greater prevalence of women that exhibit subclinical indices of disordered eating [46]. It has been suggested that some women may even present with healthy eating behaviors, but exercise at such a high volume that they are unable to compensate for exercise energy expenditure by increasing energy intake and thus, inadvertently under eat [46]. This article will provide an overview of how particular dietary strategies in which many exercising women engage to maintain low energy availability impact energy balance and the underlying physiology of menstrual cycle disturbances.
Energy Balance and Reproductive Function

In a variety of mammalian species, chronic energy deficiency may induce a reversible state of energy conservation by suppressing reproduction to preserve fuel for life-sustaining processes in the body [47]. In animal studies, reducing dietary intake by > 30% has continually led to infertility [46]. Termed functional hypothalamic amenorrhea (FHA), this has been demonstrated in an exercise model in monkeys in which amenorrhea was induced in response to an exercise-induced energy deficit. Resumption of menses occurred by increasing caloric intake and body weight while maintaining daily exercise training [48]. Several prospective studies in exercising women have reported menstrual irregularities in women in response to both decreasing energy intake or increasing exercise energy expenditure. Decreases in luteinizing hormone (LH) pulse frequency [49] as well as estrogen concentrations [50] have been exhibited in response to a diet-induced energy deficit. As well, an energy deficit induced by caloric restriction combined with vigorous exercise has been associated with decreases in estrogen as well as menstrual disturbances [51]. In the latter study, only four of 28 untrained college-aged women maintained a normal menstrual cycle during training. Those that lost weight experienced luteal phase defects as well as suppression of the pre-ovulatory LH surge. Within six months of study termination, subjects regained normal menstrual cyclicity. Notably, one study in young women showed that an energy deficit induced by diet alone suppressed LH pulse frequency more so than an equal energy deficit induced by exercise. This study also reported no change in LH pulse frequency subsequent to the exercise intervention when increased energy intake was provided to offset the exercise-induced energy deficit. Thus, in agreement with animal studies,
reproductive function is suppressed in exercising women by an energy deficit and not the stress of exercise per se.

The suppression of reproductive function in response to energy deficiency occurs at the level of the gonadotropin releasing hormone (GnRH) pulse generator when metabolic signals in the circulation or via neural pathways relay information regarding energy status to the hypothalamus (Figure 2.1.1). Suppression of GnRH pulsatility inhibits optimal LH pulse frequency signaling to the ovaries to produce estrogen and the mid-cycle LH surge that causes ovulation. However, the mechanism through which this suppression occurs is unknown. A large amount of evidence supports a key role for leptin in the modulation of reproductive function, particularly in exercising women [52]. Leptin deficiency has been associated with impaired GnRH secretion in mice where ovulation is restored with exogenous leptin administration [53]. In humans, leptin is associated with both the maintenance of LH pulsatility as well as the LH surge that causes ovulation [54]. Interestingly, it has been reported that amenorrheic women as well as those with disordered eating have lower circulating leptin concentrations as well as a blunted diurnal rhythm [14]. More recent studies suggest that gastrointestinal (GI) peptides like ghrelin may also play a role in this regulation. GI peptides are secreted from cells along the GI tract in response to nutrient ingestion, some of which are able to cross the blood brain barrier to send signals to the brain regarding nutritional status. These GI peptides may signal indirectly by way of stimulation of neuropeptides that convey GI signals regarding energy balance to GnRH neurons. For example, ghrelin binds to the growth hormone secretagogue receptor in the hypothalamus and stimulates neuropeptide Y (NPY) release from the hypothalamic arcuate nucleus. NPY is a potent orexigenic neuropeptide that
may inhibit GnRH release when estrogen concentrations are low and stimulate GnRH release when estrogen concentrations are high. Peptide YY (PYY) is a GI hormone that binds to the Y2 receptor in the arcuate nucleus and opposes the actions of ghrelin. PYY is a satiety hormone that has been shown to be elevated in exercising women with menstrual cycle disturbances; however, the role of PYY in the suppression of reproductive function is unclear. Neuropeptides that relay hormone signaling from the GI tract may act directly on GnRH neurons or indirectly by way of a recently discovered neuropeptide that is believed to be involved in the initiation of puberty and the pre-ovulatory GnRH surge, kisspeptin. Kisspeptin may act as an intermediary in these pathways transducing signals from the arcuate nucleus, and other areas that respond to changes in energy balance such as the paraventricular nucleus, to GnRH neurons [54].
Figure 2.1.1. Schematic representing metabolic signaling of energy deficiency to hypothalamic GnRH suppression.

The Female Athlete Triad

The Female Athlete Triad (Figure 2.1.2) is most prevalent in women training for sports in which low body weight is emphasized for performance or appearance; however, one or more components of the Triad have been recognized in non-aesthetic sports as well as high school athletes and non-competitive exercising women [46]. A high prevalence exists when considering women diagnosed with one or more components of the Triad, and the prevalence varies depending on the population of interest. The
percentage of exercising women that have been recognized as having disordered eating has been estimated to be as high as 62 % [55]. However, the American College of Sports Medicine emphasizes that women may also become energy deficient in the absence of disordered eating [46]. Some exercising women may be unable to compensate for a high volume of exercise energy expenditure and thus, inadvertently fail to increase energy intake to adequately meet the needs of energy expended. However, studies have not been performed to determine the existence and prevalence of inadvertent under eating. The prevalence of menstrual irregularities in exercising women has been documented as high as 69 % [46]. Most studies utilize self-reported menstrual history to document menstrual disturbances which may underestimate the prevalence as some women may have a regular cycle length and thus, report normal menses, but may have incidence of more subtle disturbances. Menstrual cycle disturbances have been documented ranging from subtle disturbances such as luteal phase defects (LPD) and anovulation to oligomenorrhea (cycle length of 36 - 90 days) and amenorrhea. LPD may not be detected as cycle length may be within a normal range (26 - 35 days); however, may manifest as a short luteal phase of < 10 days or inadequate luteal phase where progesterone does not attain concentrations high enough to sustain the health of the corpus luteum. One study carefully characterized urinary estrogen, progesterone and LH across 2 to 3 consecutive menstrual cycles in 67 exercising women and demonstrated that 50 % of cycles were abnormal and 33.7 % were amenorrheic (Figure 2.1.3) [56].
Figure 2.1.2. Schematic representation of the Female Athlete Triad. Adapted from De Souza et al. 2004 with permission (See Appendix for license agreement).
Energy Availability

Dietary strategies utilized by exercising women to achieve low body weight and low body fat promote an energy deficit and consequently, lower energy availability. This may lead to reproductive suppression and the menstrual cycle disturbances detailed above. Even though exercising women require an increased energy intake to match
energy expenditure, there have been several reports that they consume the same amount, or even less, than their sedentary counterparts [57]. Energy availability, operationally defined as dietary energy intake minus exercise energy expenditure, refers to the amount of dietary energy that remains for other bodily functions after accounting for exercise energy expenditure, and is typically quantified relative to fat free mass (FFM) [58]. Optimal energy availability is needed to maintain reproductive function where 45 kcal/kgFFM is considered to be the energy availability at which healthy energy balance and menstrual function are sustained. Energy availability can be lowered by decreasing energy intake, increasing exercise energy expenditure, or both [46]. Dietary strategies to lower energy availability, explained below, are all avenues through which energy availability may be lowered to a level at or below which menstrual cycle disturbances occur. It has been suggested that an energy availability below 30 kcal/kgFFM may suppress reproductive function. To their detriment, exercising women in a chronic energy deficit may be weight stable and as a result, presume that they are in a healthy energy balance. However, several other indications of energy conservation have been demonstrated such as suppressed resting metabolic rate (RMR) which may decrease total energy expenditure and endocrine abnormalities like low triiodothyronine and elevated circulating concentrations of ghrelin [59]. Consequently, body weight might be stable during a period where energy availability is low.

**Dietary Strategies used by Exercising Women to Maintain Low Body Weight and Low Body Fat**

**Low Body Fat: Impact on the Menstrual Cycle**

Several dietary strategies are utilized by exercising women to maintain low body weight and low body fat ranging from clinically diagnosed eating disorders to subclinical
disordered eating and potentially inadvertent under eating (Table 2.1.1). Many exercising women may utilize one or more strategies to maintain low body weight for more aesthetic reasons as opposed to sport performance. Compared to men, twice as many women perceive themselves to be overweight. Those that are actively attempting to lose weight are even higher; the proportion of which increase as body mass index decreases such that nearly nine times the number of lean women as lean men are engaged in some strategy to lose weight.
### Table 2.1.1. Summary of dietary strategies used to maintain low EA and associated menstrual cycle disturbances

<table>
<thead>
<tr>
<th>Dietary Strategy</th>
<th>Description</th>
<th>Associated Menstrual Disturbance(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eating Disorders (AN, BN, ED-NOS)</strong></td>
<td>Clinically diagnosed psychiatric conditions. Most severe form of energy restriction.</td>
<td>Amenorrhea is a diagnostic criterion</td>
</tr>
<tr>
<td><strong>Disordered Eating</strong></td>
<td>Subclinical disorders including energy restriction not necessary for health or performance</td>
<td>Subtle (LPD, anovulation) to severe (oligo- and amenorrhea) menstrual disturbances</td>
</tr>
<tr>
<td><strong>Dieting</strong></td>
<td>Energy restriction to lower caloric intake that is not necessarily associated with aberrant eating or body image attitudes</td>
<td>Subtle (LPD, anovulation) to severe (oligo- and amenorrhea) menstrual Disturbances</td>
</tr>
<tr>
<td><strong>Food Avoidance</strong></td>
<td>Avoidance of particular foods that are high in fat, i.e., red meat, ice cream, dairy products, etc. to restrict energy intake. Considered a ‘red flag’ for potential risk of disordered eating.</td>
<td>Subtle to severe menstrual disturbances; due to energy restriction and not lack of a specific macronutrient</td>
</tr>
<tr>
<td><strong>Vegetarianism</strong></td>
<td>Avoiding high-fat foods like meat to restrict energy intake. Considered a ‘red flag’ for potential risk of disordered eating.</td>
<td>Anovulation, suppressed estrogen and progesterone concentrations</td>
</tr>
<tr>
<td><strong>Low Energy Dense Diets</strong></td>
<td>Increasing fruit/vegetable intake and decreasing fat intake to decrease dietary energy intake</td>
<td>Subtle (LPD, anovulation) to severe (oligo- and amenorrhea) menstrual disturbances</td>
</tr>
<tr>
<td><strong>Aberrant Eating Behavior</strong></td>
<td>High drive for thinness and/or high cognitive restraint may lead to or exacerbate</td>
<td>Subtle (LPD, anovulation) to severe (oligo- and amenorrhea) menstrual disturbances</td>
</tr>
<tr>
<td><strong>Inadvertent Under Eating</strong></td>
<td>Exercise may inadvertently induce appetite suppression and lead to accidental energy restriction</td>
<td>No evidence of menstrual disturbances, more research is needed</td>
</tr>
</tbody>
</table>
Eating Disorders

Eating disorders the most severe form of energy restriction to maintain low energy availability and are clinically diagnosed psychiatric conditions in which individuals may be hospitalized if health is at risk. AN is characterized by restrictive eating in which individuals have a distorted body image, viewing themselves as overweight and are afraid of weight gain despite being < 85% expected weight for age and height. Individuals suffering from BN tend to have a normal body weight and repeat cycles of overeating or binging and then purging or engaging in other compensatory behaviors such as fasting or excessive exercise. Eating disorders not otherwise specified (ED-NOS) are a category of eating disorder in which most, but not all criteria for AN or BN are met. Eating disorders are considered the most severe form of energy restriction and pathogenic weight control and consequently, are more likely associated with the most severe menstrual disturbance, amenorrhea, which is currently a diagnostic criterion for AN.

Disordered Eating

More recently, disordered eating has been included in describing the arm of the Female Athlete Triad related to energy availability suggesting that the Triad exists along a spectrum of subclinical disturbances that may or may not lead to a clinically diagnosed eating disorder. Indices of disordered eating include, but are not limited to, dieting that is unnecessary for health, sport performance or appearance, avoiding certain foods or food groups, vegetarianism/veganism, binge-eating and purging, use of laxatives and/or diuretics and excessive exercise or exercising despite injury [55].
Dieting

Dieting may lower energy intake and/or lead to insufficient macro- or micronutrient intake during a period where energy requirements are higher in exercising women than their sedentary counterparts. It has been suggested that energy intake below 1800 kcal/day (7430 kJ/day) is inadequate to meet the needs of training, especially in adolescent women that are still growing [57]. Additionally, exercising women have increased carbohydrate and protein requirements. In comparison to the recommended dietary allowances (RDA), upwards of 5 – 10 grams/kg body weight and 1.2 – 1.4 g/kg body weight (RDA = 0.8 g/kg), respectively, are recommended. Not meeting these requirements can lead to poor performance, slower recovery and increase risk of injury [57]. When exercising women restrict energy intake and do not adequately meet the needs of exercise energy expenditure, the incidence of menstrual disturbances increases [51]. Subtle to severe menstrual disturbances have been reported in exercising women that restrict energy intake [46]. Consequently, larger and more chronic energy deficits may lead to more severe menstrual disturbances such as oligomenorrhea and amenorrhea. To that end, recovery of regular, ovulatory menstrual cycles may be a product of increasing caloric intake to match expenditure and restore a healthy energy availability. As well, time to recovery may depend the amount of calories supplemented to offset the energy deficit [48].

Food Avoidance: Low-Carbohydrate and Low-Fat Dieting

Some exercising women will avoid certain foods (i.e. red meat) or macronutrients like carbohydrate or fat to maintain low energy intake; however, proper balance of macronutrient intake is essential in maintaining performance and health [60]. Avoiding
certain foods or macronutrients, for example avoiding red meat or maintaining a low carbohydrate diet, may lead to energy restriction in exercising women that have an increased energy requirement. No particular macronutrient impacts the menstrual cycle more so than another; however, inducing an energy deficit by way of food avoidance may lead to menstrual cycle disturbances if energy intake does not adequately meet the needs of exercise energy expenditure. Though, there is no macronutrient that will impact the menstrual cycle more than another, low carbohydrate diets, in endurance athletes particularly, may be extremely detrimental with regard to skeletal muscle metabolism during exercise. Carbohydrates maintain blood glucose concentrations and are a main substrate for skeletal muscle metabolism during exercise, and they are used to replace muscle glycogen during post-exercise recovery [60]. Fat is the only macronutrient with no recommended value for intake; however, restricting dietary fat intake may be detrimental because, in addition to energy and use as a main substrate in skeletal muscle metabolism during prolonged exercise, it provides essential elements of cell membranes and aids in absorption of fat-soluble vitamins, A, D, E and K [60].

**Vegetarianism**

Vegetarians tend to be leaner and lighter than non-vegetarians and exercising women may utilize a vegetarian diet to restrict energy intake by avoiding high-fat foods such as meat. These individuals may report wanting to ‘eat well’ though the underlying reason of becoming vegetarian may be energy restriction to induce weight loss [61]. Thus, being or becoming a vegetarian is seen as a ‘red flag’ or risk factor for disordered eating [46, 60]. Adopting a vegetarian diet has been associated with menstrual disturbances. One study [62] randomized normal-weight women to either a vegetarian or
non-vegetarian diet. In this study, both groups lost an average of 1 kg body weight each week over a six week intervention period; however, seven of the nine women eating the vegetarian diet were reported to have anovulatory menstrual cycles characterized with suppressed estrogen, progesterone and LH concentrations, whereas seven of nine women eating the non-vegetarian diet maintained regular ovulatory menstrual cycles.

**Low Energy Dense Diets**

Vegetarian diets tend to be low in dietary energy density (caloric content of food per gram weight; kcal/gram). Lowering dietary energy density by increasing fruit and vegetable intake and/or lowering fat intake has been demonstrated to be a key strategy by which successful weight loss and weight loss maintenance occurs [30]. Low energy density diets have been reported in exercising women with menstrual disturbances as a dietary strategy to maintain low energy intake [63]. In this study, consumption of vegetables was greater in exercising women with menstrual cycle disturbances when compared to exercising, ovulatory control subjects. Additionally, when compared to exercising ovulatory control subjects, fasting PYY concentrations, a potential biomarker of satiety, were higher in women with menstrual cycle disturbances indicating that satiety may be enhanced in exercising women with menstrual disturbances that consume a diet low in energy density (Figure 2.1.4). Interestingly, subjects in this study had high intake of low- or no-calorie condiments which may have been used to make bland foods taste better without adding caloric value. Additional studies are required to confirm that lowering dietary energy density is associated with menstrual disturbances.
**Figure 2.1.4.** Bar graphs comparing the A. dietary energy density and B. fasting total PYY between Ovulatory (Ov) control and Exercise Associated Menstrual Disturbance (EAMD) subjects. Data are reported as mean ± SD; *p < 0.05. Reprinted from Reed et al. 2011 with permission (See Appendix for license agreement).

**Aberrant Eating Behavior Phenotypes: Dietary Restraint and Drive for Thinness**

Though dieting tends to be an initial avenue through which disordered eating manifests, environmental and social cues such as pressure from coaches or teammates to maintain low body weight, low self-esteem, and negative eating attitudes like high drive for thinness, dietary restraint or body dissatisfaction may also contribute to, or exacerbate
disordered eating [46]. Surveys like the Three-Factor Eating Questionnaire and the Eating Disorder Inventory are utilized to screen for such behaviors. One study [64] demonstrated that restraint influenced relative energy intake (energy intake after accounting for exercise energy expenditure) subsequent to acute exercise in normal weight, exercising women. The study demonstrated a greater decrease in relative energy intake from rest to exercise in restrained compared to unrestrained women; suggesting that eating behavior may be one factor involved in the modulation of energy intake subsequent to acute exercise. There is also evidence that biological factors and genetics may predispose an athlete to disordered eating [46]. For example, reduced satiety and suppressed secretion of cholecystokinin, a satiety hormone, has been reported in patients with BN and may be one factor contributing to the uncontrollable drive of BN patients to binge. It has been suggested that the etiology of eating disorders may be genetically linked due to higher prevalence rates of AN and BN in fraternal twins and first degree relatives of those who have AN. It is interesting to note that aberrant eating behaviors typically associated with disordered eating and eating disorders could potentially be utilized as surrogate markers indicating energy deficiency and consequent reproductive dysfunction. High drive for thinness (DT) has been associated with suppressed RMR, an indication of energy conservation, as well as oligomenorrhea and amenorrhea [65]. Additionally, one study demonstrated that women with high dietary restraint exercised more, were more likely to be vegetarian, have a history of eating disorders, were more likely to be actively trying to lose weight and reported irregular menstrual cycles [66]. Thus, indices of disordered eating, like high dietary restraint or high DT, may be an indication of energy restriction and could potentially be easily assessed in exercising women to detect or prevent poor
dietary habits that may lead to energy deficiency and consequently reproductive dysfunction.

**Inadvertent Under Eating**

Inadvertent under eating may also be an avenue through which exercising women exhibit low energy availability. It has been suggested that some exercising women may be unable to compensate for high volumes of exercise energy expenditure and inadvertently fail to increase energy intake to match energy expended through exercise. Exercising women with menstrual disturbances exhibit elevated circulating concentrations of ghrelin [59], the only hormone known to stimulate hunger and increased food intake. Increases in ghrelin are thought to be part of a homeostatic feedback mechanism involved in increasing body weight back to a predetermined ‘set point.’ As well, increases in circulating ghrelin concentrations may be signaling to the hypothalamus to suppress reproduction by way of suppressed LH secretion and pulsatility [54]. However, elevated circulating concentrations of PYY, a satiety hormone that has been shown to be elevated in exercising women with amenorrhea [5], may be opposing the impact of increased ghrelin on hunger and food intake and promoting chronically low energy intake.

In addition to chronic changes in hormone profiles, recent research suggests that changes in appetite and gastrointestinal hormone responses induced by acute exercise may contribute to a suppressive effect of exercise on appetite. Acute bouts of exercise may suppress appetite and lead to a non-compensatory response in relative energy intake. The mechanism through which exercise suppresses energy intake is not well understood, but may potentially be one avenue through which exercising women inadvertently restrict
energy intake. Recent literature also suggests that GI hormones involved in appetite regulation and energy balance exhibit an anorexigenic hormone profile during and subsequent to exercise. Suppressed concentrations of acylated ghrelin have been observed during aerobic exercise where after ad libitum energy intake was not different from a no exercise condition and thus, individuals did not compensate for the energy deficit induced by exercise [67]. Exercise-induced suppression of acylated ghrelin appears to be transient; however, the increases elicited in the satiety hormones PYY and glucagon-like peptide I may be sustained through the post-exercise period [68]. Thus, the prolonged participation of women in endurance exercise may promote a cumulative anorexigenic hormone profile, despite chronic elevations in ghrelin, and aid in the chronic suppression of energy intake in these women.

Aberrant eating behavior phenotypes and alterations in GI hormone profiles are potential contributing factors to chronic energy restriction observed in exercising women. However, integration of these signals occurs through the brain where a complex network of internal (i.e. GI hormones) and external (i.e. smell, taste, etc.) influences may modulate homeostatic and hedonic responses to food. The hypothalamus is a central region in the brain involved in the homeostatic regulation of energy balance. Neuroimaging studies have demonstrated that hunger stimulates, whereas satiety inhibits, activation of the hypothalamus. The insula, in particular, is one brain region involved in food reward responses that plays a role in connecting the neural networks of the hypothalamus to other brain areas signaling hunger and may respond to acute exercise more so than other food reward brain regions [69]. Recent research has focused on these brain regions to begin to determine how aberrant patterns of food reward, hunger and
satiety signals may differ among obese and lean individuals; however, little is known about the effect of acute bouts of exercise on food reward and associated brain regions. Preliminary evidence utilizing functional magnetic resonance imaging (fMRI) suggests that exercise suppresses the activation of brain regions associated with food reward. In response to acute exercise, Evero et al. [70] observed reduced neuronal responses in the insula and orbitofrontal cortex which is consistent with reduced pleasure of food, incentive motivation to eat and anticipation and consumption of food. Further investigation of the suppressive effect of exercise on brain regions associated with food reward is needed, particularly in exercising women that may be at risk for low energy availability and thus menstrual disturbances.

**Meal Timing and Meal Related Gut Peptides Responses as Energy Signals**

Lastly, it is interesting to note that ghrelin, as well as several other gastrointestinal hormones involved in hunger and satiety responses, not only respond to chronic changes in energy status, but also to decreases in acute energy intake. Higher concentrations of PYY are associated with caloric and macronutrient content of food as well as meal timing across the day [20]. Alternatively, ghrelin has been shown to be negatively associated with similar meal-related parameters [13]. As well, ghrelin and PYY oppose the actions of one another on the GI tract and recent data from our lab shows that they may be reciprocally involved in the modulation of one another in the circulation (Figure 2.1.5). It is interesting to posit that these short term GI signals that respond to more subtle, daily fluctuations in meal timing and caloric quantity of food eaten across a single day may be important modulators of menstrual function. In support of this hypothesis, recent data from our lab has shown that in female collegiate soccer players has demonstrated that
energy availability may fluctuate across the day depending on energy content of specific meals (Figure 2.1.6). Over time, signaling from day-to-day energy deficits created by long periods of low caloric intake throughout the day may accumulate and lead to chronic reproductive suppression. Consequently, future studies could potentially focus on whether meal timing and caloric content regimens that maintain optimal energy availability on a daily basis would be associated with menstrual function.

Figure 2.1.5. 24h composite profile illustrating an inverse association of (●) total PYY and (□) ghrelin. Data are expressed as mean ± SEM; p < 0.05 (Unpublished).
**Conclusions**

In conclusion, menstrual cycle disturbances in exercising women have been largely attributed to a chronic energy deficit and thus, regardless of the avenue through which exercising women restrict energy intake, it is likely that any avenue that will induce an energy deficit may suppress reproductive function. Studies suggest that the mechanism through which chronic energy deficits suppress reproduction may be through altered metabolic hormone signaling. In particular, ghrelin may to be involved in the suppression of LH secretion and pulsatility; however, further investigation as to this mechanism is warranted. It may be advised that exercising women take care in planning
meals across the day so that caloric and macronutrient content of meals as well as meal timing are optimal to sustain a healthy energy availability and prevent induction of a chronic energy deficit that may lead to reproductive suppression.

**Summary Points**

- Chronic energy deficiency may suppress reproductive function to preserve energy for life-sustaining physiological functions; however, the mechanism through which this occurs is unclear.

- Hormones such as ghrelin and leptin may signal a state of low energy availability to the hypothalamus where GnRH secretion is suppressed. GnRH suppression inhibits the pulsatile release of luteinizing hormone which results in inhibition of folliculogenesis and ovulation.

- Exercising women may be at an increased risk of developing one or more components of the Female Athlete Triad which consists of three interrelated clinical conditions i.e., low energy availability, menstrual disturbances and low bone mass. The etiology of the Triad has been attributed to chronic energy deficiency induced by inadequate energy intake to compensate for exercise energy expenditure.

- Low energy availability may lead to suppressed reproductive function. Both conditions may increase risk of low bone mass and/or fracture.

- Dietary strategies to maintain low energy availability include eating disorders, disordered eating and inadvertent under eating.

- Eating disorders are clinically diagnosed psychiatric conditions that represent the most severe form of energy restriction where amenorrhea has been considered a diagnostic criterion.
• Disordered eating represents many subclinical manifestations, all of which impact the menstrual cycle by way of the induction of a chronic energy deficit.

• High drive for thinness and high dietary restraint may lead to or exacerbate disordered eating and may be indications of energy deficiency in exercising women with menstrual disturbances.

• Exercise may acutely suppress appetite, elicit an anorexigenic gastrointestinal hormone profile and alter food reward responses leading to inadvertent under eating; however, more studies are needed to address this effect in exercising women.

• Short term GI signals that respond to more subtle, daily fluctuations in meal timing and caloric quantity of food eaten across a single day may be important modulators of menstrual function.

• Exercising women may need to plan meals across the day so that caloric and macronutrient content of meals as well as meal timing are optimal to sustain a healthy energy availability and prevent induction of a chronic energy deficit that may lead to reproductive suppression.
Part 2

The Regulation of Synthesis, Secretion and Actions of Peptide YY (PYY)

Synthesis, Secretion, and Activation of PYY

Synthesis and Activation of PYY

Peptide tyrosine-tyrosine (PYY) is a 36-amino-acid peptide hormone that is synthesized and co-secreted along with glucagon-like peptide 1 (GLP-1) from endocrine L cells in the distal sections of the small intestine, mainly the ileum, colon and rectum [2, 71]. PYY was isolated from porcine intestine in 1980 and is a member of the pancreatic polypeptide family to which pancreatic polypeptide (PP) and neuropeptide Y (NPY) also belong [72, 73]. PYY synthesis occurs as a result of activation of the G protein-coupled receptor (Gs) and subsequent activation of adenylate cyclase, the enzyme that converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), thereby increasing intracellular cAMP and activating the protein kinase A (PKA) signaling cascade [74]. One study demonstrated that synthesis may also be stimulated by the calcium-dependent protein kinase C (PKC) signaling cascade [74].

PYY undergoes COOH-terminus amidation which is essential for biological activity. As well, two amino acids, tyrosine and proline, are cleaved at the NH2-terminus from the secreted and biologically inactive form of PYY, PYY_{1-36}. Cleavage of tyrosine and proline forms the more biologically active and predominant postprandial form of PYY, PYY_{3-36}, by way of dipeptidyl peptidase IV (DPP-IV), a cell surface enzyme ubiquitously expressed on endothelial and epithelial cells [75, 76].
Modulation of the Synthesis and Secretion of PYY

Neural Factors Involved in the Modulation of PYY Secretion

A neural mechanism, potentially vagal stimulation, is believed to stimulate the release of PYY. This has been proposed because appearance of PYY in the circulation occurs within 15 minutes of meal ingestion, prior to nutrient exposure at L cells in the distal gut from which PYY is secreted [77]. This neural reflex mechanism may be region-specific, occurring exclusively in the duodenum, whereas colonic release of PYY is likely a result of direct stimulation of L cells by intraluminal nutrients [78]. Figure 2.2.1 demonstrates a hypothesized model of the neural regulation of PYY secretion [78]. Evidence to support neural stimulation of PYY release has been demonstrated by several studies. Fu-Cheng et al. [79] infused a liquid meal into rat duodenum that induced PYY release from the distal intestine prior to the meal reaching that area of the gut. Authors concluded that it is likely that vagal efferent neuronal projections releasing acetylcholine (Ach) activate duodenal cholinergic receptors which consequently stimulate the synthesis and secretion of PYY distally in the ileum and colon. In support, vagal stimulation has been shown to induce PYY release and blocking Ach receptors with atropine blocked food-induced release of PYY in the dog [80] as well as the pig [81]. Interestingly, basal and food-induced release of PYY has also been elevated in response to truncal vagotomy demonstrating that vagal efferent neurons may also play a role in the inhibition of PYY secretion [80]. One possible explanation of the disparity in results may be that mechanisms differ depending on the prandial state, i.e. fasting or postprandial states. Burdyga et al. [82, 83] have suggested that vagal afferent neurons may have the ability to differentially express hormone receptors. For example, ghrelin receptor expression is
increased in the fasted state and thus, signaling to the brain a need to increase food intake and satiety.

hormone receptor, i.e. CCKR-1 and Y2R to which cholecystokinin (CCK) and PYY bind, respectively, expression increases while in the fed state to signal to the brain regarding satiety and thereby leading to meal cessation.

Additionally, it may be that more than one secretory mechanism is contributing to PYY release. Electrical stimulation of the splanchnic nerve in dogs [80] and administration of a β-adrenergic agonist [84] have both induced PYY release. It is thus likely that several mechanisms occur to induce PYY release; however, further exploration of the mechanism by which PYY is released and whether these mechanisms occur simultaneously or in succession is needed.
Caloric and Macronutrient Content of Meals

The Response of PYY to Dietary Fat

Figure 2.2.2 demonstrates the proposed mechanism by which intraluminal nutrient exposure stimulates the secretion of PYY from endocrine L cells in the ileum and colon [78]. In general, it seems as though a broad range of nutrient stimuli in the colon elicit increases in PYY whereas few stimuli, i.e., oleic acid and bile acids (aid in micelle formation and dietary fat digestion) provoke increases in circulating PYY from the duodenum and ileum [85]. In dogs [86] and rats [87], infusion of oleic acid, a monounsaturated fatty acid, elicited subsequent increases in plasma PYY, particularly when infused into the ileum and to a lesser extent in the colon and duodenum. Short-chain fatty acids seem to elicit the greatest response in PYY in the rat colon [88] and little to no effect has been observed in the ileum [79]. In the pig, Cuche et al. [89] demonstrated that short-chain fatty acids elicited increases in PYY and the increases observed in PYY were similar in both innervated and denervated ileum. As well, one study demonstrated that this response was not inhibited by hexamethonium, an antagonist of Ach receptors which are involved in the vagal stimulation response [79]. Thus, it is likely that intraluminal fat exposure directly stimulates the secretion of PYY [79]. As well, the secretory profile of PYY in response to luminal fat exposure differs depending on the intestinal region in which the fat exposure occurs.

In humans, it has been demonstrated that PYY is secreted in proportion to meal energy content as well as macronutrient content, where high-fat or high-protein meals stimulate greater PYY secretion than high-carbohydrate meals [2-4]. Higher circulating concentrations of PYY have been observed in response to an isocaloric meal of fat
compared to that of protein or carbohydrate [2, 90]. One study in obese individuals demonstrated that a low-carbohydrate, high-fat diet provided for one week elicited significant increases in postprandial PYY concentrations that were 55 % greater than postprandial PYY concentrations subsequent to a week-long low-fat, high carbohydrate diet [91]. As well, Feinle-Bisset et al. [92] performed 120-minute infusions of intraduodenal fat with or without a lipase inhibitor (tetrahydrolipstatin; THL), to prevent the breakdown of fat, in healthy young men to determine whether luminal fat exposure is an essential stimulus for PYY secretion. Results of this study demonstrated that when fat was co-infused with THL the fat-induced increases in PYY were abolished. Authors concluded that the stimulation of PYY secretion is dependent upon fat digestion. Thus, in humans as well as animals, it is likely that PYY secretion depends, at least in part, upon intestinal lumen exposure to dietary fat.
The Response of PYY to Dietary Carbohydrate

Results have varied with regard to the stimulation of PYY secretion by carbohydrate. In rats, no change in plasma PYY was observed when physiological doses of intraluminal glucose were administered at isoosmolar concentrations to that administered of oleic acid [79]. Supra-physiological doses of glucose have elicited increases in PYY in isolated, vascularity perfused rat ileum and colon [88]. As well, hyper-perfusion of maltose, a glucose disaccharide, into the colon elicited increases in PYY; however, that same

**Figure 2.2.2.** Hypothetical model of the regulation of PYY secretion in endocrine L cells in the distal intestine. Thickness of line indicates potency of the stimulus. Dotted lines indicate regulatory pathways thought to exist. “+” and “−” indicate stimulatory and inhibitory action, respectively. AC, adenylate cyclase; PKA, protein kinase A; m, muscarinic receptor. Reprinted from Onaga 2002 with permission (See Appendix for license agreement).
dose of maltose administration had no effect on PYY secretion in the ileum [93]. In humans, glucose infusions had no effect on PYY secretion when infused into the cecum and colon [94]. Thus, PYY may increase in response to carbohydrate exposure in a region-specific manner in some species; however, the doses utilized in the studies noted herein were supra-physiological in nature and it is unlikely that the distal portions of the intestine from which PYY is secreted would encounter such doses.

**The Response of PYY to Dietary Protein**

Infusion of peptone solutions into isolated rat ileum has elicited increases in PYY whereas infusion of an amino acid mixture had no little to no impact on PYY secretion in the ileum, but did increase PYY secretion from the colon [79, 88, 95]. In humans, infusion of casein solution, a protein found in cow’s milk, in either the cecum or colon had no effect on plasma PYY concentrations [94]. However, another study demonstrated that a high-protein meal elicited greater increases in total PYY than isocaloric high-fat or high-carbohydrate meals in obese as well as lean individuals [3]. This same study exhibited similar results with regard to protein-mediated stimulation of PYY secretion in mice in response to a single meal and also demonstrated that a long-term high-protein diet reduced weight gain and elicited increases in fasting and postprandial PYY secretion. Thus, proteins may stimulate region-specific PYY secretion that is not elicited by hydrolyzed amino acids and the effect of protein stimulation of PYY release may depend on the specific type of protein ingested.

**Endocrine Factors Involved in the Modulation of PYY Secretion**

In addition to vagal and nutrient stimulation of PYY synthesis, several endocrine and neuroendocrine factors may also stimulate PYY secretion (Figure 2.2.2)
demonstrating that a complex network of neural, chemical and endocrine factors is involved in the modulation of PYY secretion. CCK, in particular, is a hormone secreted from I cells in the duodenum that aids in fat and protein digestion by stimulating the release of digestive enzymes and bile from the pancreas and gall bladder, respectively. CCK seems to play a major role in the stimulation of PYY secretion. Brubaker et al. [74] incubated cultured rat intestinal cells that were immunoreactive for PYY with CCK demonstrated no effect of CCK isolated intestinal cells. However, in contrast, one study demonstrated that a meal comprised of fat and a separate intravenous infusion of CCK both stimulated PYY release in dogs [90]. Pre-treatment with a CCK receptor antagonist (L-364,718) abolished the effects of both the meal and CCK infusion on PYY release. As well, Lin et al. [96] also demonstrated that duodenal administration of oleate, a monounsaturated omega-9 fatty acid, in combination with a CCK antagonist (devazepide) in dogs suppressed the distal gut release of PYY when compared to oleate infusion without devazepide. Another study demonstrated that CCK receptor blockade abolished the effect of long-chain fatty acid stimulation of PYY secretion demonstrating that PYY secretion may be modulated by CCK receptor activation [97]. As well, Y2 receptor, the receptor to which PYY preferentially binds, expression on vagal afferent neurons was increased when vagal afferent neurons were cultured with CCK [98]. Consequently, it is likely that CCK plays a major role stimulating the release of PYY in the distal gut by way of proximal gut release of CCK and signaling from CCK receptor activation. As well, Y2 receptor expression on vagal afferent neurons and consequently, PYY signaling from the gastrointestinal (GI) tract to the brain may be mediated through intracellular signaling cascades subsequent to CCK receptor activation.
In addition to CCK, other hormones have been shown to be involved in the modulation of PYY secretion. Infusion of supraphysiological doses of GLP-1, which is co-secreted with PYY from L cells and is involved in glucose-stimulated insulin release, suppressed circulating concentrations of PYY in normal weight men [99], which may suggest negative feedback paracrine actions of GLP-1 on L cells. Gastric distension, a factor believed to be involved in the satiety response to a meal, has been shown to have no influence the secretion of PYY [99-101]. However, growth factors that stimulate GI mucosal growth such as insulin-like growth factor-1 (IGF-1) and transforming growth factor-α (TGF-α) may stimulate the secretion of PYY [101]. Interestingly, growth hormone (GH), a key factor in the regulation of IGF-1 production, has been shown to suppress PYY secretion in rats [102].

Cumulatively, the endocrine factors involved in the modulation of PYY secretion are numerous and it is likely that there is no solitary factor that plays a dominant role in regulating PYY secretion. It is likely that the factors mentioned herein are all involved in the modulation of PYY secretion and each of these factors contributes, in part, to the overall control and modulation of PYY secretion.

The Role of PYY in Acute Energy Balance: Single Meal Responses and the Diurnal Rhythm

Fasting and Single Meal Responses of PYY

PYY is characterized by low fasting concentrations that begin to increase within 15 – 30 minutes of meal initiation. This initial rise in PYY is posited to be due to vagal stimulation as this occurs prior to nutrients reaching the region of the gut where PYY-secreting L cells are located (discussed in the previous section). Continued release of
PYY occurs as nutrients reach L cells which are highly concentrated in the distal ileum and colon. PYY concentrations reach a peak 1 - 2 hours postprandially and may remain elevated for up to six hours [2]. Upon attainment of peak concentrations, PYY decreases toward fasting concentrations until another meal is initiated [2] (Figure 2.2.3).

PYY is believed to be one factor involved in meal termination and has consequently been identified as a satiety hormone. Several studies suggest more frequent meals across the day may allow for beneficial metabolic effects as well as greater 24-hour satiety [103-105]. Changes in PYY across the day and/or in response to meals and meal timing may contribute to this effect. However, the diurnal rhythm of PYY has yet to be fully characterized under normal physiological conditions, or in relation to meal timing and meal energy or macronutrient content of meals. Several groups have reported the 24-hour profile of PYY; however, these studies were in reference to pathophysiological conditions and obtained samples at time intervals where capturing meal responses may be compromised [6, 106-108]. Whether altering meal timing would affect peak PYY concentrations is unknown. Therefore, it may be advantageous to explore what modulates the secretion of PYY and how the modulation of PYY secretion impacts satiety across the day.
Figure 2.2.3. Changes in circulating concentrations of total PYY (pg/ml) in response to a single meal (n = 11). Data are reported as mean ± SEM (Unpublished).

Central and Peripheral Actions of PYY

The PYY Receptor

There are five Y receptors, Y1 through Y5, to which the PP family (PP, PYY and NPY) preferentially binds. PYY\textsubscript{1-36} preferentially binds to the Y1 receptor whereas PYY\textsubscript{3-36}, the more biologically active form of PYY, preferentially binds to Y2 and Y5 receptors with the highest affinity. The Y receptor family is characterized as a seven transmembrane domain receptors coupled to a G\textsubscript{i} protein that results in the inhibition of adenylate cyclase which inhibits the conversion of ATP to cAMP and as a result, inhibits the activation of PKA.

PYY Activation of the Hypothalamic Arcuate Nucleus

It is hypothesized that peripherally secreted hormones modulate energy balance through integration within the hypothalamic arcuate nucleus (ARC). The role of PYY in
the regulation of energy balance likely occurs through several pathways. Circulating PYY is secreted from L cells in the ileum and colon and is able to freely cross the blood-brain-barrier in a nonsaturable mechanism due to the ability of NH$_2$-terminus to interact with phospholipid cell membranes [109]. In support of this direct mechanism, it has been demonstrated that administration of PYY$_{3-36}$ into the ARC reduces foot intake in a dose-dependent manner [19] and intra-arcuate injection of a Y2 receptor antagonist abolishes this effect [110].

Within the ARC, PYY preferentially binds to the Y2 receptor on orexigenic NPY/Agouti-related protein (AgRP) neurons that co-express the growth hormone secretagogue receptor (GHS-R) to which ghrelin binds. Ghrelin receptor activation stimulates NPY release and induces a cascade of neuroendocrine events that lead to stimulation of energy intake. One mechanism through which this occurs is NPY-induced inhibition of pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons that release α-melanocyte stimulating hormone (α-MSH), a potent anorexigenic neuropeptide [111, 112]. As well, AgRP is an antagonist of the melanocortin receptors, MC-3 and MC-4, to which α-MSH binds [113] and thus, competitively inhibits the binding and anorexigenic actions of α-MSH. PYY binds to, and activates the Y2 receptor on NPY/AgRP neurons which inhibits the release of NPY and AgRP. PYY-induced inhibition of NPY release allows for the activation of POMC/CART neurons and subsequent release α-MSH. As well, inhibition of NPY/AgRP neuronal activity releases the competitive inhibition of AgRP on MC-3 and MC-4 receptors allowing for α-MSH signaling and ultimately decreasing food intake and increasing energy expenditure [100, 114, 115]. In support of this mechanism, Riedeger et
al. [22] demonstrated in male Wistar rats that PYY injection inhibited activation of ghrelin-sensitive NPY/AgRP neurons within the ARC.

In addition to the direct actions of PYY within the ARC, PYY may modulate energy balance signaling through Y2 receptor activation on vagal afferent neurons [116]. Vagal afferent neurons project to the nucleus of the solitary tract (NTS) and area postrema (AP) which then transmit these peripheral signals to the hypothalamus; however, the intracellular signaling cascades and intermediate neuropeptides through which this occurs are unknown. Vagotomy or the ablation of the AP both suppress the ability of PYY to inhibit food intake [110, 117] and thus, the mechanism through which PYY inhibits food intake depends upon, at least in part, vagal afferent neuronal signaling cascades.

**Peripheral Actions of PYY**

**Actions of PYY on the Gastrointestinal Tract: Gastric Acid Secretion and Gut Motility**

PYY acts as an “ileal brake” by inhibiting gastric acid and intestinal secretion as well as gastric emptying and motility to promote the absorption of nutrients [118-121]. Infusion of PYY to doses similar to that observed postprandially in dogs suppressed gastric acid secretion [23]; however, doses twice that were necessary to delay gastric emptying [122] and elicit an inhibition of pancreatic enzyme secretions [123]. One study demonstrated in rats that colonic administration of short-chain fatty acids resulted in reduced colonic motility as well as PYY release [124]. When PYY was infused to concentrations similar to that observed in response to short-chain fatty acids exposure, colonic motility was reduced in similar manner. As well, immunoneutralization of
circulating PYY (animals were provided PYY anti-serum) abolished the effects of short-chain fatty acids on colonic motility demonstrating that PYY likely mediates reductions in colonic motility observed in response to short-chain fatty acids.

In humans, PYY infusion to physiological doses similar to postprandial concentrations has inhibited gastric acid and pepsin secretion and delayed GI transit time; however, a dose three times that was unable to alter pancreatic or biliary secretions [121]. Another study demonstrated that GI transit time as well as gastric emptying were delayed in response to physiological as well as supraphysiological doses of PYY when compared to saline infusions in a population of healthy men [125]. Cumulatively, results suggest that PYY plays a modulatory role in digestion and absorption by slowing gastric acid secretion, gastric emptying and motility. Consequently, the slowing of gastric emptying and motility promotes signaling of sensations from the stomach during food intake with regard to satiation [126] which may be one mechanism through which PYY is involved in the postprandial satiety response.

**PYY as a Satiety Hormone**

In addition to the role of PYY in slowing gastric acid secretions and GI motility, more recent literature has begun to characterize PYY as a satiety hormone and its role in postprandial satiety and meal cessation. Circulating concentrations of PYY increase within 15 minutes of meal initiation and continue to rise until peak concentrations are reached 1 – 2 hours postprandially. PYY may remain elevated in the circulation for several hours and has a half-life of 8 – 10 minutes [127] and thus, may have a more prolonged impact than other GI hormones with a shorter half-life such as GLP-1 which has a half-life of only 1 – 2 minutes [128]. Peripheral injection of PYY into rats has
produced reduced food intake [129]; effects that are similar to that of centrally administered PYY (explained above). Intraperitoneal injection of PYY into rats has also reduced food intake in response to twice daily injections of PYY for seven days [19]. As well, the knock out of the Y2 receptor to which PYY preferentially binds abolishes the effects of PYY on food intake and elicits an obese phenotype [19].

In healthy, normal weight humans, PYY infusions to physiological doses have suppressed fasting hunger scores when compared to infusions of saline. As well, food intake at a buffet meal two hours after the termination of PYY infusions was significantly lower and this food intake suppression lasted through 12 hours after termination of PYY infusions [19]. In obese individuals, low circulating concentrations of PYY have been reported [7, 8] concomitant with reduced satiety in response to an isocaloric meal ingested by lean counterparts [7]. As well, obese individuals require greater food consumption than their lean counterparts during a meal to elicit similar postprandial increases in PYY [7]. Though circulating concentrations of both fasting and postprandial PYY are lower in obese individuals, it has been demonstrated that food intake is suppressed at a buffet meal subsequent to physiological doses of PYY infusions [8]. As well, 90-minute infusion of PYY to physiological doses in obese and lean individuals elicited decreases in cumulative 24-hour food intake [8]. Consequently, it is likely that obese individuals are not PYY resistant per se, but that inhibition of PYY occurs at the level of synthesis and secretion. PYY is likely a major contributing factor in postprandial satiety and low circulating PYY observed in obese individuals may contribute to the pathogenesis of obesity.
PYY in the Regulation of Other Endocrine Factors in Central and Peripheral Tissues

In addition to the role of PYY acting within the hypothalamic ARC as a short-term satiety signal and potential role in long-term energy balance, PYY may also be a factor involved in signaling throughout the body. PYY may signal in centrally located tissues like the pituitary gland impacting the secretion of hormones like thyrotropin (TSH) and luteinizing hormone (LH) and peripheral tissues such as pancreatic and adipose tissues which may impact insulin homeostasis and lipolysis, respectively.

The Role of PYY in the Modulation of the Hypothalamic-Pituitary-Adrenal Axis

PYY may exert actions on centrally located tissues such as the pituitary gland where it may aid in modulating the hypothalamic-pituitary-adrenal (HPA) axis. One study demonstrated that incubation of PYY<sub>3-36</sub> with rat pituitary cells elicited a dose-dependent decrease in TSH, but did not alter the responsiveness of TSH to thyrotropin-releasing hormone [130]. In contrast, in vivo doses of PYY elicited an increase in serum TSH in fasted, but not fed rats and these doses of exogenous PYY had no impact on circulating thyroxine (T4) or triiodothyronine (T3) [130]. In addition to the potential central action of PYY on pituitary cells, Neri et al. [131] demonstrated that administration of PYY to medullary cells within rat adrenal gland stimulated the production of aldosterone, but not corticosterone. As well, adrenocorticotropic hormone-stimulated aldosterone and corticosterone release were inhibited by PYY administration to isolated rat zona glomerulosa cells [131]. Authors concluded that discrepancies between in vivo and in vitro experiments may be a result of the likelihood that there are numerous factors involved in the regulation of the HPA axis in vivo. Thus, PYY may
play a modulatory role within the hypothalamic-pituitary-adrenal axis; however, further investigation is required to clarify the role for PYY and the mechanism through which PYY acts to aid in the regulation of the HPA axis.

**The Role of PYY in the Modulation of Reproductive Function**

Little is known as to whether PYY plays a role in the modulation of reproductive function; however, recent literature suggests that PYY may promote healthy reproductive function through modulation of hypothalamic-pituitary-gonadal axis signaling. It has been suggested that hormones secreted from the GI tract, such as PYY, may signal regarding energy status to the hypothalamus, which contains control centers for both energy balance and reproduction, to potentially suppress reproduction during times in which food resources may be scarce, thereby preserving energy stores for life-sustaining body systems. The proposed mechanism by which this occurs is the suppression of gonadotropin-releasing hormone (GnRH) by metabolic cues; however, the metabolic cues that are involved in the suppression of GnRH are unknown [47]. LH is a gonadotropin released from the pituitary gland in response to GnRH pulses from the ARC and is considered a proxy indication of reproductive health, i.e., suppression of LH pulsatility is an indication of suppressed reproductive function. In male and female rats, Fernandez-Fernandez et al. [132] have recently characterized the Y2 and Y5 receptors, to which PYY preferentially binds, on pituitary cells. Authors demonstrated that PYY administration to pituitary cells enhanced LH secretion in a dose-dependent manner; however, this effect was not observed in response to intraperitoneal injection of PYY. Other studies have demonstrated that NPY, an orexigenic neuropeptide that opposes the anorexigenic actions of PYY, inhibited estrous behavior in Syrian hamsters [133, 134].
As well, Xaio et al. [135] confirmed the presence of PYY expression in placental and fetal membranes that persisted from 9.5 weeks through pregnancy in humans; however, support for a modulatory role of PYY in pregnancy was not tested. Thus, support for the role of PYY in reproductive function has been provided in animal models, but further research is needed to clarify a modulatory role for PYY in reproductive function in humans.

**The Role of PYY in Glucose and Insulin Homeostasis**

Studies have exhibited a role for PYY in insulin and glucose homeostasis [136-138]. Bottcher et al. [136] demonstrated that PYY inhibits glucose-stimulated insulin secretion from pancreatic islet cells in vivo, but had no effect on basal insulin or glucose concentrations in mice. Another study demonstrated that infusion of PYY\textsubscript{3-36} may also improve insulin sensitivity in insulin-resistant mice fed a high-fat diet [139]. It has been demonstrated that PYY knockout mice that do not produce either circulating form of PYY are similar in glucose tolerance to control mice, but that insulin is markedly increased for the duration of a glucose tolerance test [140]. Increases observed in insulin were a result of insulin hyper-secretion from islets of Langerhans cells in response to high glucose concentrations [141]. In contrast, Batterham et al. [8] infused PYY in obese and lean subjects to determine the impact of PYY infusion on food intake and appetite. Food intake, appetite and fasting and postprandial ghrelin (described below) were all suppressed in both lean and obese humans; however, PYY infusions had no impact on circulating insulin. Thus, it is likely that, in addition to the inhibitory role of PYY in slowing gastric acid secretion and motility, that PYY may also inhibit pancreatic insulin secretion in response to elevations in blood glucose; however, the mechanism through
which this occurs is unclear and may differ among species. It is likely that actions occur through activation of the Y1 receptor and not through the Y2 or Y5 receptors. The Y1 receptor has been discovered on beta cells that produce insulin [142] and as well, the actions of PYY on insulin regulation is more potent in response to PYY\textsubscript{1-36}, which preferentially binds to the Y1 receptor, when compared to PYY\textsubscript{3-36} which preferentially binds to the Y2 and Y5 receptors [138].

**The Role of PYY in Fat Metabolism**

PYY has been shown to be involved in the metabolism of fat. As a hormone secreted predominantly in the postprandial state where inhibition of lipolysis and stimulation of lipogenesis occurs, it is no surprise that studies have demonstrated that PYY inhibits lipolysis in both animals and humans. In isolated fat cells of both dogs and humans, Valet et al. [143] demonstrated that PYY administration inhibited lipolysis in a dose-dependent manner. As well, administration of pertussis toxin to block the G-protein through which PYY signals abolished the PYY-induced inhibition of lipolysis. One study in humans infused PYY into samples of adipose tissue obtained from differing body regions and demonstrated inhibited lipolysis in response to PYY more so in femoral adipose tissue when compared to pericolonic or mammary adipose tissues [144]. As well, one study demonstrated that peripheral infusion of PYY in humans increased metabolic rate (kJ/day), serum free fatty acid concentrations and lowered respiratory quotient (RQ) demonstrating increased fatty acid metabolism [15]. Thus, PYY likely plays a role in the postprandial metabolism of fat by way of several mechanisms to contribute to the digestion and absorption as well as storage of fat.
The Role of PYY in Energy Expenditure

Most of the research conducted to date regarding PYY has been performed to determine the effects of PYY on the GI tract and satiety. However, some studies have also suggested a potential role for PYY in the modulation of energy expenditure \cite{15, 16}. The role of PYY in energy expenditure is unclear as evidenced by several disparities in the current literature which may be the result of varying study populations or methodological discrepancies \cite{5, 7, 9, 17}. Guo et al. \cite{17} detected a negative correlation between fasting PYY and resting metabolic rate (RMR) as well as RQ and postprandial peak PYY in a cohort of obese and lean individuals. In contrast to this negative correlation between PYY and RMR, Sloth et al. \cite{15, 18} peripherally infused PYY in lean and obese men and demonstrated increases in energy expenditure (kJ/day) which may suggest a positive association. However, results from this study also demonstrated that increased fat oxidation, as indicated by lowered RQ, also resulted from PYY infusions which supports the negative correlation between PYY and RQ observed by Guo et al. \cite{17} and may suggest that PYY is involved in the up-regulation of fatty acid metabolism. As well, one study in normal weight, premenopausal women demonstrated a positive correlation between PYY and postprandial energy expenditure \cite{145}. Whether an association exists between changes in circulating PYY and indices of energy expenditure, such as 24-hour or postprandial energy expenditure, remains to be elucidated. It may be possible that greater circulating concentrations of PYY are acting on hypothalamic Y2 receptors where PYY binds and inhibits NPY/AgRP release and subsequent orexigenic effects. Y2 receptor binding is also thought to activate POMC neurons enhancing anorexigenic effects and increasing energy expenditure \cite{114, 115}. 
To the contrary, changes in resting metabolism or postprandial energy expenditure may be driving a greater secretion of PYY; however, a mechanism by which metabolism may stimulate PYY secretion has yet to be elucidated. Thus, further research is necessary to determine the relationship between PYY and energy expenditure.

The Role of PYY in Chronic Energy Balance

The function of PYY in acute energy balance as a satiety hormone seems to be well established; however, in humans, the role of PYY in chronic energy balance is unclear compared to what has been shown in animal studies. PYY is one of many satiety hormones that has recently been shown to be involved in the modulation of chronic energy balance in several elegantly performed animal studies [3, 8, 19]. Studies involving animal models have demonstrated that chronic infusion of PYY induces weight loss [3, 8, 19]. Batterham et al. [19] demonstrated that twice daily peripheral injections of PYY into rats over seven days inhibits food intake and reduces weight gain. As well, the knock down of the receptor to which PYY preferentially binds, the Y2 receptor, leads to weight gain and induces obesity in mice and rats [19]. Infusion of PYY has induced weight loss as well as a reduction in adiposity in normal weight rodents [146], diet-induced obese rodents [147, 148] and monkeys [149]. However, the translation of these findings to humans has yet to be exhibited. To date much of the work in humans has been observations of single-meal responses in pathophysiological conditions [4, 6-9] where normal physiological responses have been perturbed. Thus, studies in individuals without chronic disease may aid in our understanding of the role of PYY in the etiology of disease and further research in humans is needed to clarify the role of PYY in the modulation of chronic energy balance.
Fasting as well as postprandial PYY concentrations have been shown to be suppressed in obese [7, 8, 150, 151] and elevated in individuals experiencing a chronic energy deficit, i.e. exercise-associated energy deficiency [5] or anorexia nervosa [150, 151]. However, it is unknown as to whether individuals have developed aberrant profiles of circulating PYY as a result of the development of their respective pathophysiological states or if suppressed or elevated concentrations of PYY predispose an individual to these pathophysiological conditions. With weight loss in obesity, or weight gain in the instance of energy deficiency, i.e. anorexia nervosa, circulating PYY does not necessarily return to concentrations observed in normal weight counterparts [38]. Though scarce, some research has been conducted to measure chronic changes in PYY in response to changes in body weight. Unfortunately, no definitive association has been elucidated as studies have shown varying results where decreases [37-39], no change [40] or even increases [36, 152] in PYY in response to weight loss have been observed. Le Roux et al. [7] demonstrated that PYY secretion is suppressed in obese subjects in comparison to lean counterparts, but that these individuals remain sensitive to the effects of PYY on energy intake subsequent to PYY infusion. Thus, it may be that obese individuals are predisposed to low circulating PYY and women experiencing chronic energy deficits, such as that observed in anorexia nervosa, are predisposed to high circulating PYY. Consequently, these predispositions to low or high circulating PYY may contribute to the pathophysiological states in which they reside.
The Association between PYY and Ghrelin

Hypothalamic Associations between PYY and Ghrelin

The gut-brain axis is a complex interwoven cascade of enteroendocrine hunger and satiety hormones signaling to and from the brain. Signaling occurs when hormones secreted from the GI system in response to a meal enter circulation and either cross the blood-brain-barrier to bind to receptors [109] and/or signal through receptors on vagal afferent neurons [80, 153]. PYY and ghrelin may be involved, not only in the modulation of energy intake, but in the modulation of one another, possibly through opposing actions within the ARC [22].

PYY Y2 receptor activation and signaling has been previously discussed above. Briefly, PYY preferentially binds to the Y2 receptor on NPY/AgRP neurons located in the ARC. PYY receptor activation on ghrelin-sensitive NPY/AgRP neurons inhibits the release of the NPY and AgRP. The suppression of NPY release allows for the activation of POMC neurons and subsequent release of the anorexigenic neuropeptide, α-MSH, which promotes decreases in food intake and increased energy expenditure [100, 114, 115]. As well, AgRP release is suppressed which releases the competitive inhibition of AgRP on MC-3 and MC-4 receptors to which α-MSH binds.

Ghrelin is able to cross the blood-brain-barrier and bind to the GHS-R on NPY/AgRP neurons in the ARC. Subsequent to receptor activation, NPY is released inducing a cascade of neuroendocrine events that lead to stimulation of energy intake and inhibition of the release of α-MSH from POMC neurons [111]. This association between ghrelin and PYY has been recently demonstrated by Riedeger et al. [22] in male Wistar rats where PYY injection inhibited activation of ghrelin-sensitive neurons within the AN.
Thus, PYY and ghrelin inhibit the anorexigenic and orexigenic actions, respectively, of one another within the hypothalamic ARC.

**Peripheral Associations between PYY and Ghrelin**

Studies have previously proposed that the underlying mechanism by which ghrelin and PYY modulate energy balance operates at the level of the hypothalamus where these hormones cross the blood brain barrier [109] and bind to receptors in the arcuate nucleus [22]. However, these hormones are secreted from cells along the GI tract in the periphery and secretion is stimulated (PYY) or inhibited (ghrelin) by caloric as well as macronutrient content of meals [2, 13, 20]. PYY slows GI motility and suppresses gastric acid secretion [23] whereas ghrelin stimulates those same actions [24]. One study infused ghrelin and demonstrated an attenuation of the effects of PYY on gastric acid secretion and motility [25]. Thus, ghrelin and PYY may oppose the actions of one another on digestion and absorption within the GI tract. As well, receptors for ghrelin and PYY are expressed on a number of tissues in the periphery including the adrenal gland [71], adipose tissue [144] and pituitary gland [71]. Therefore, merely focusing on hypothalamic regulation of these hormones may only provide insight into a narrow realm thereby limiting the study of what these hormones may modulate, including each other, in the periphery. Consequently, measurement of peripheral signals may be a superior indication of total body regulation of the endocrine control of energy balance.

Although ghrelin and PYY may reciprocally inhibit the actions of one another on the GI tract, the mechanism through which ghrelin and PYY may modulate the actual secretory profiles of one another in the periphery is unknown [26]. Ghrelin decreases, whereas PYY increases postprandially and thus, the reciprocal association may
exclusively be a result of the incidental regulation by way of nutrient ingestion. However, evidence to support a regulatory association between ghrelin and PYY was demonstrated when Batterham et al. [8] showed that fasting concentrations of ghrelin decreased significantly and, as well the pre-prandial rise in ghrelin was abolished two hours subsequent to infusion of PYY in obese and lean men and women. Though it is noteworthy that these infusions resulted in physiological doses of the hormone, infusions were only performed for 90 minutes and thus, only encompassed the span of one subsequent meal. Another study by Feinle-Bisset et al. [92] showed that infusion of intraduodenal fat, a macronutrient known to stimulate PYY secretion [2, 90], also suppressed circulating concentrations of ghrelin in young, healthy men. In light of this, it is attractive to hypothesize that ghrelin and PYY may be directly involved in modulating the secretion of one another in a negative feedback manner in the periphery. However, few studies have examined the association between ghrelin and PYY in the peripheral circulation [8, 25, 154]. And, to date, no study has demonstrated an association between ghrelin and PYY in the circulation beyond single, 90-minute infusions of PYY or single meal responses. Thus, it may be advantageous to determine if an association exists in humans over an entire 24-hour period and as well, if such an association changes in response to changes in energy balance.

The Association between Psychosocial Variables and PYY

The Association between Behavioral Variables and Physiological Outcomes

Circulating GI hormones such as ghrelin and PYY may be related to psychosocial measures of eating behaviors such as dietary restraint (the tendency to consciously restrict food intake to control body weight), disinhibition (the loss of control over eating...
in response to emotional or social cues) and tendency toward hunger, which are assessed by the Eating Inventory [155]. Some studies have linked physiological mechanisms of gut hormones involved in the control of energy balance to psychosocial variables [41-45]. For example, high dietary restraint has been associated with high circulating concentrations of ghrelin in healthy, non-obese individuals [41]. One study exhibited an association between PYY and disinhibition such that subjects with high disinhibition tended to have a blunted PYY response, but failed to show an association between PYY and restraint [43]. Additionally, a recent study by Langlois et al. [45] demonstrated that ghrelin was positively correlated to scores for hunger independent of BMI. These studies have begun to relate behavioral to physiological measures; however, the interactions between these factors have not been determined. It is attractive to posit that altered physiological states, such as suppressed concentrations of PYY, may be modulating behavioral changes, such as increases in dietary restraint, to offset biological abnormalities that may influence meal-related hunger and satiety. However, more research is needed to clarify if a connection exists between physiological and behavioral systems.

**Conclusions**

PYY is a postprandial satiety hormone that is involved in energy balance and plays a role in the inhibitory response of several postprandial GI functions such as gastric acid secretion, motility and glucose-stimulated insulin homeostasis. The release of PYY is likely a result of both vagal and nutrient stimulation where dietary fat and protein stimulate the synthesis and secretion of PYY more so than carbohydrate. Both circulating forms of PYY, PYY\textsubscript{1-36} and PYY\textsubscript{3-36}, the more biologically active form, exert
actions at differing levels of potency depending on which actions are in question and thus, both circulating forms of PYY are physiologically relevant. PYY may be involved in the modulation of the secretion of other endocrine factors from central and peripheral tissues such as the pituitary gland and GI, adipose and reproductive tissues and is thus, likely a hormone involved not only in GI function, but the regulation of the functions of multiple body systems. The mechanisms through which PYY acts on both central and peripheral tissues are currently unclear, but depend upon Y receptor activation. Further study is needed to clarify the pathways through which PYY exerts its actions and how these actions modulate central and peripheral control of physiological systems.
Chapter 3


Abstract

PYY may play a role in modulating satiety and energy expenditure; increasing postprandially PYY has largely been studied in single-meal responses. The diurnal rhythm of PYY and its role in energy balance have not been fully characterized.

PURPOSE Characterize features of the diurnal rhythm of PYY and determine its role in regulating energy balance. METHODS This study was a cross-sectional analysis of 11 subjects in whom 24 h repeated blood sampling was conducted at baseline of a larger prospective study. Breakfast (B), lunch (L), dinner (D), and a snack (S) occurred between 0900h-1900h. Total PYY was assayed every h from 0800-1000 h, 20 min from 1000-2000 h and h from 2000-0800 h. PYY variables included total AUC, postprandial peaks, and 24h mean. Energy balance variables included energy intake, RMR, RQ, and NEAT. RESULTS PYY postprandial peaks were significantly higher than fasting (p < 0.05). 24 h peak PYY occurred after L and was significantly higher than all other peaks (p < 0.05). A cubic curve function accounted for most of the variance in PYY (R² = 69.9 %, p < 0.01). Fasting PYY (0800 h) correlated with postprandial peaks at B (r = 0.77, p = 0.01), L (r = 0.71, p = 0.01), and D (r = 0.65, p = 0.03). The only significant association between PYY and energy expenditure was that RMR (kcal/24h) correlated with 24 h mean PYY (r = 0.71, p = 0.013) and total AUC (r = 0.69, p = 0.019).

CONCLUSION PYY displays a meal-driven diurnal rhythm and is correlated to RMR, a
major contributor to energy expenditure. Thus, PYY varies in accordance with energy content and RMR, supporting a role for PYY in energy balance modulation.

**Introduction**

Peptide YY (PYY) is secreted from L-cells in the gut where it slows digestion, acting as an “ileal brake” to increase absorption of nutrients [1]. PYY\textsubscript{1-36}, cleaved to PYY\textsubscript{3-36} via dipeptidyl peptidase, is secreted in proportion to energy and macronutrient content, where high-fat or high-protein meals stimulate greater PYY secretion than high-carbohydrate meals [2-4]. PYY reaches peak concentrations 1-2 hours postprandially and aids in meal cessation [2] and has thus been identified as a satiety hormone.

Fasting as well as postprandial PYY concentrations have been shown to be depressed in obese and elevated in energy deficient women [5-8]. Findings regarding the effect of weight loss/gain on PYY are conflicting as well as its role in energy balance [5, 9, 17, 37, 39, 152, 156]. To date much of the work in humans has been observations of single-meal responses in pathophysiological conditions [4, 6-9] where normal physiological responses have been perturbed. Thus, studies in individuals without chronic disease may aid in our understanding of the role of PYY in the etiology of disease.

The diurnal rhythms of hormones like ghrelin and leptin have been characterized in terms of responses to meal timing [10-12], energy content of meals [13], and the impact of energy deficiency [13, 14]. The diurnal rhythm of PYY has yet to be fully characterized under normal physiological conditions, or in relation to meal timing and meal energy or macronutrient content of meals. Studies of single-meal responses do not
address whether PYY responses change across the day due to time of day or as nutrient intake accumulates.

Studies have also suggested a potential role for PYY in the modulation of energy expenditure (EE) [15, 16]. In the hypothalamus, PYY binds to inhibitory Y2 receptors (Y2R) in the arcuate nucleus (AN) where neuropeptide Y (NPY) and proopiomelanocortin (POMC) neurons are located. PYY binds to Y2R on NPY neurons inhibiting orexigenic NPY secretion. This inhibition releases inhibition of POMC neurons resulting in greater POMC activation and thus, secretion of anorexigenic hormones (α-MSH) and increasing EE [100, 114, 115]. Guo et al. [17] found a negative correlation between fasting PYY and resting metabolic rate as well as respiratory quotient and postprandial peak PYY. Additionally, Sloth et al. [15, 18] peripherally infused PYY and showed increases in EE (kJ/day) as well as fat oxidation (lowered RQ) in lean and obese men.

The purpose of this study was to characterize features of the diurnal rhythm of PYY and to explore the role of PYY in energy balance in normal weight premenopausal women. We hypothesized that meal energy content and timing would be key components in eliciting the 24-hour profile of PYY and that PYY would correlate positively to indices of energy expenditure.

**Subjects and Methods**

**Experimental Design and Subjects**

This study was part of a larger prospective study designed to assess the effects of energy restriction on metabolism and reproductive function. We examined the 24-hour profile of total PYY and energy balance parameters in subjects during the baseline
period. PYY was measured in blood samples from 11 subjects who completed 24-hour blood sampling and anthropometric and energy balance measurements on days 2 - 7 of the follicular phase. Non-smoking, healthy, non-exercising (< 1 hour/week purposeful exercise) women ages 18 - 30 years of age with a body weight of 48 - 73 kg, 15 – 30 % body fat and BMI between 18 - 25 kg/m² were included. Exclusion criteria included any evidence of disordered eating or history of an eating disorder, loss/gain of a significant amount of weight (± 2.3 kg) in the past year, or use of hormonal contraceptives or medication that may alter metabolic hormones. Each subject signed an informed consent approved by the Biomedical Institutional Review Board of The Pennsylvania State University.

**Screening**

Subjects provided information regarding demographics, medical history, menstrual history, and physical activity along with eating attitudes questionnaires. A fasting blood sample was obtained between 0600 - 1000 h for analysis of a complete blood count, basic chemistry panel, and to rule out abnormal pituitary function or metabolic diseases. Psychological stability and the absence/risk of eating disorders were established in an interview under the supervision of a clinical psychologist. Subjects met with a general clinical research center (GCRC) registered dietician to ensure absence of aberrant dietary habits and suitability for a controlled feeding study. Documentation of 2-3 ovulatory menstrual cycles prior to the study was performed with measurements of mid-luteal phase serum progesterone and the mid-cycle urinary LH surge (First Response, Tambrands, Inc.).
Anthropometrics

Hydrostatic weighing was performed after correcting for residual lung volume to determine body composition. Body density was used to calculate body composition using the Brozek equation [157]. Body weight was measured on the same day with subjects wearing shorts, a tee shirt (without shoes) and recorded to the nearest 0.01 kg.

Energy Balance Parameters

Resting Metabolic Rate

Resting metabolic rate (RMR) was measured using a ventilated hood system between 0600-1000h following an overnight fast. Subjects lay in the supine position for 20-30 minutes to acclimate to the room temperature and testing procedures; the hood was placed over the subject’s head for 30 minutes. Expired air was measured every minute for carbon dioxide and oxygen concentration using a carbon dioxide analyzer (URAS4, Hartmann & Braun, Frankfurt, Germany) and a paramagnetic oxygen analyzer (Magnos 4G, Hartmann & Braun). The values for minutes in which steady state was achieved were averaged to calculate RMR (kcal/day), determined using the Weir equation [158], and respiratory quotient (RQ).

Physical Activity Expenditure

To determine 24-hour EE subjects wore a tri-axial activity monitor (AM) (RT3 accelerometer, Stayhealthy, Monrovia, CA) 24 hours/day for one 7-day period to assess the energy cost of all non-purposeful exercise EE (kcal). The AM was worn on the left hip for three weeks of baseline and was not worn during showering/bathing. Subjects recorded weekly AM logs which identified all types of activity and when the monitor was taken off. As all subjects were sedentary at baseline and thus, did not accumulate EE
from exercise thus, RMR (kcal/24h) and the average daily EE from the AM were added together to determine total 24-hour EE.

**Free-Living Dietary Intake**

Three-day diet logs were completed by each subject and consisted of records from two weekdays and one weekend day. Subjects recorded any and all energy intake from food and beverage for the entire day. Data were analyzed in Nutritionist Pro (First Data Bank, Indianapolis, IN) by GCRC registered dieticians.

**Determination of Baseline Energy Needs**

Caloric intake required to maintain weight for each subject (baseline energy needs) was calculated based on the sum of EE from the measurement of 24-hour RMR and AM kcal. The prescribed diet was prepared by the GCRC metabolic kitchen and then provided for a 7-day calibration period during baseline where subjects were weighed daily, and ±100 kcal adjustments were made if body weight fluctuated by more than ±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.

**24-hour Assessment of PYY Diurnal Rhythm**

All subjects were tested in the follicular phase (days 2 - 7) at least one week after the calibration period. Subjects were instructed to abstain from exercise or caffeine ingestion 24 hours prior to the test and to fast as of 2000 h the night prior. Subjects arrived at the GCRC at 0730h the day of testing where they remained in a supine position with their upper body 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 2500 rpm. Serum aliquots were transferred to storage tubes and stored in a -80 degree freezer until 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 % carbohydrates, 30 % fat, and 15 % protein and consisted of three meals and a snack prepared for 0900 h (breakfast), 1200 h (lunch), 1800 h (dinner) and 2100 h (snack). Dinner consisted of 503 ± 0.4 kcal the remainder of which was distributed between breakfast (416 ± 30 kcal), lunch (486 ± 26 kcal) and the snack (66 ± 4 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 EE due to inactivity associated with bed rest. Meal composition was as follows: breakfast: 48 % carbohydrate, 32 % fat, and 20 % protein; lunch: 54 % carbohydrate, 32 % fat, and 14 % protein; and dinner: 55 % carbohydrate, 30 % fat, and 15 % protein. A snack comprised of 95 % carbohydrate, 2 % fat, and 4 % protein was provided at 2100 hours. 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.

**PYY Radioimmunoassay Analysis**

Serum samples were assayed for total circulating PYY hourly from 0800-1000h, every 20 minutes from 1000-2000h and hourly from 2000-0800h. PYY was assayed using a radioimmunoassay (Millipore, Billerica, MA). The sensitivity of the assay was
10 pg/mL and the intra-assay and inter-assay coefficients of variation were 2.9 % and 7.1 %, respectively.

**Data Analysis**

Fasting PYY was designated as 0800h. Postprandial peak PYY was defined as the highest PYY concentration (pg/ml) obtained after meal administration and prior to subsequent meal administration (1-2 hours postprandially). PYY nadirs were determined to be the lowest PYY pre-prandial concentration before each meal. Twenty-four-hour mean PYY was the average of all PYY concentrations (pg/ml) observed in the 24-hour analysis. Total area under the curve (AUC) was calculated by the trapezoidal rule. Four-hour blocked AUC was defined as meal response AUC for the time between 0800-1200h, 1200-1600h, 1600-2000h, and 2000-0000h to capture the breakfast, lunch, dinner and snack responses, respectively and 0000-0400 and 0400-0800h to capture the nocturnal AUCs. Meal rises were defined as the change in PYY that occurred from meal administration to the peak postprandial PYY concentration (pg/ml). Lastly, 24-hour peak PYY was considered the highest concentration of PYY (pg/ml) obtained in the entire 24-hour sampling period.

**Statistical Analyses**

To determine the presence of a diurnal rhythm, 24-hour PYY profiles were analyzed using ANOVA with repeated measures to determine differences in meal-related postprandial peaks across the day. When main effects were detected, *post hoc* analyses were performed by means of paired sample t-tests employing the Bonferroni correction factor. Regression curve-fit analysis was also employed to determine what, if any, curvilinear function best fit the 24-hour profile of PYY. To determine the relationship
between fasting PYY and meal-related PYY features, Pearson correlations were performed to assess the relationship between fasting PYY (pg/ml) and 24-hour mean PYY, AUC variables, and meal peaks/nadirs. Pearson correlations were calculated to determine the association between PYY and energy balance parameters such as body composition variables, 24-hour sampling meal energy and macronutrient content, 3-day dietary intake and macronutrient composition, RMR, and RQ. A p-value of less than 0.05 was considered statistically significant. Data are reported as mean ± SEM and all analyses were performed using SPSS software (Version 18.0; Chicago, IL).

Results

Subjects

Subject demographics are shown in Table 3.1. Subjects were healthy normal weight, pre-menopausal women. Subjects were sedentary at baseline (< 1 h purposeful exercise per week) and had remained weight stable (no significant weight loss/gain ± 2.3 kg) for at least one year prior to the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>20 ± 0.6</td>
<td>18 – 24</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.7 ± 1.4</td>
<td>159.4 – 175.3</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>58.5 ± 1.4</td>
<td>51.1 – 66.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.1 ± 0.6</td>
<td>18.4 – 23.9</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>27.0 ± 1.6</td>
<td>19.7 – 36.8</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>15.9 ± 1.2</td>
<td>10.7 – 22.2</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>42.6 ± 1.2</td>
<td>37.4 – 49.6</td>
</tr>
</tbody>
</table>
PYY Diurnal Rhythm

Figure 3.1 illustrates the 24-hour profile of total circulating PYY. PYY exhibits a meal-driven diurnal rhythm characterized by elevated postprandial concentrations after every meal that were significantly higher than fasting PYY. The 24-hour peak PYY concentration, observed after lunch, was significantly higher than fasting PYY and all other postprandial peaks across the day (Figure 3.1B). This was likely due to an additive effect of breakfast and lunch calories combined which was correlated to the lunch postprandial peak PYY concentration ($r = 0.64, p = 0.04$). Presumably, the between-meal duration of 3 hours between breakfast and lunch allowed for a greater lunch postprandial PYY peak than the 6 hours between lunch and dinner even though energy content of lunch and dinner was not significantly different ($p = 0.52$). Additionally, despite the difference in absolute PYY concentrations between lunch and dinner, the meal rises at lunch and dinner were not significantly different ($p = 0.90$).

To further characterize the diurnal rhythm of PYY, total AUC was partitioned into 4-hour blocks of time to illustrate meal-related AUC. The lunch response AUC from 1200-1600h was significantly higher than the breakfast response AUC from 0800-1200h ($p = 0.001$). Also, the nocturnal AUC blocks (0000-0400h; $p = 0.003$ and 0400-0800h; $p = 0.006$) were significantly lower than the 0800-1200h AUC block. No significant difference was found when comparing the dinner response AUC (1600-2000h; $p = 0.54$) to the breakfast response. Figure 3.1C displays the result of a regression curve-fit analysis where a cubic curvilinear function accounted for a significant amount of the variance in 24-hour PYY ($R^2 = 69.9\%$, $p < 0.01$).
Figure 3.1. A. Composite 24-hour profile of total PYY (pg/ml) illustrating meal administration time points B. Fasting and postprandial meal peaks of total PYY (pg/ml). C. Regression curve-fit analysis of 24-hour total PYY (pg/ml) illustrating a cubic curve function (n=11). Data are expressed as mean ± SEM; p < 0.05
Fasting PYY and 24 hour Diurnal Rhythm

Fasting PYY (pg/ml; 0800h) correlated with postprandial meal peaks at breakfast (r = 0.77, p = 0.01), lunch (r = 0.71, p = 0.01), and dinner (r = 0.65, p = 0.03) as well as total AUC (r = 0.86, p = 0.001) and 24-hour mean PYY (r = 0.83, p = 0.002). Figure 3.2A depicts the relationship between fasting PYY and total AUC and the 24-hour mean PYY. Additionally, fasting PYY correlated significantly with total energy intake (kcal/day; r = 0.63, p = 0.04) provided the day of 24-hour blood sampling as well as combined energy intake from breakfast and lunch (kcal; r = 0.64, p = 0.03) and total energy intake from carbohydrate (g; r = 0.63, p = 0.04), fat (g; r = 0.64, p = 0.03) and protein (g; r = 0.60, p = 0.05). These same meal energy content variables also showed significant, positive correlation with total AUC and 24-hour mean PYY. (Table 3.2)
**Figure 3.2.** A. Scatter plot illustrating Pearson correlations between fasting PYY (pg/ml) and AUC (pg/ml x day) and 24-hour mean PYY (pg/ml). B. Scatter plot illustrating Pearson correlations between RMR (kcal/24hr) and 24-hour mean PYY (pg/ml) and AUC (pg/ml x day). p < 0.05
Table 3.2. Pearson correlation coefficients between PYY and meal-related parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fasting</th>
<th>AUC</th>
<th>24hr Mean</th>
<th>B Peak</th>
<th>L Peak</th>
<th>D Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 24-hour (kcal)</td>
<td>0.63*</td>
<td>0.70*</td>
<td>0.71*</td>
<td>0.68*</td>
<td>0.63*</td>
<td>0.67*</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>0.64*</td>
<td>0.70*</td>
<td>0.72*</td>
<td>0.69*</td>
<td>0.64*</td>
<td>0.68*</td>
</tr>
<tr>
<td>Total CHO (g)</td>
<td>0.63*</td>
<td>0.73*</td>
<td>0.74*</td>
<td>0.72*</td>
<td>0.69*</td>
<td>0.64*</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>0.60*</td>
<td>0.67*</td>
<td>0.69*</td>
<td>0.68*</td>
<td>0.61*</td>
<td>0.69*</td>
</tr>
<tr>
<td>Breakfast (kcal)</td>
<td>0.37</td>
<td>0.49</td>
<td>0.52</td>
<td>0.54</td>
<td>0.43</td>
<td>0.65*</td>
</tr>
<tr>
<td>Lunch (kcal)</td>
<td>0.72*</td>
<td>0.70*</td>
<td>0.69*</td>
<td>0.62*</td>
<td>0.64*</td>
<td>0.48</td>
</tr>
<tr>
<td>B + L (kcal)</td>
<td>0.64*</td>
<td>0.70*</td>
<td>0.72*</td>
<td>0.70*</td>
<td>0.64*</td>
<td>0.69*</td>
</tr>
<tr>
<td>Dinner (kcal)</td>
<td>-0.25</td>
<td>-0.26</td>
<td>-0.25</td>
<td>-0.17</td>
<td>-0.29</td>
<td>-0.15</td>
</tr>
<tr>
<td>Snack (kcal)</td>
<td>0.34</td>
<td>0.44</td>
<td>0.46</td>
<td>0.40</td>
<td>0.43</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*p<0.05; B=breakfast, L=lunch, D=dinner, N=nocturnal

Day of 24-hour Sampling

PYY concentrations expressed in a variety of ways were significantly correlated with the energy content from all meals across the day. Table 3.2 illustrates correlations between total kcal, macronutrient content and meal energy content and PYY peaks, nadirs, fasting PYY, AUC and 24-hour mean concentrations across the day.

PYY and Energy Balance

Table 3.3 depicts energy balance parameters for both energy intake as well as expenditure. Energy intake is reported from 3-day diet logs in terms of average total daily intake (kcal) as well as macronutrient content (grams). EE variables include total daily caloric expenditure (kcal/24h), RMR (kcal/24h and kcal/kgFFM/24h), RQ and EE from AM (kcal/24h).
Table 3.3. Energy balance parameters

<table>
<thead>
<tr>
<th>Dietary Intake (3-day Diet Logs)</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Daily Intake (kcal)</td>
<td>1806 ± 124</td>
<td>1315 – 2459</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>240.8 ± 15.7</td>
<td>193.5 – 324.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>61.7 ± 6.0</td>
<td>28.1 – 89.0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>69.2 ± 5.1</td>
<td>42.5 – 92.5</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>4.1 ± 2.0</td>
<td>0.02 – 18.6</td>
</tr>
<tr>
<td>CHO (%)</td>
<td>51.9 ± 1.6</td>
<td>41.3 – 58.0</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>33.5 ± 1.7</td>
<td>27.1 – 37.8</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>13.0 ± 0.7</td>
<td>8.0 – 16.0</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>1.5 ± 0.8</td>
<td>0.01 – 7.5</td>
</tr>
</tbody>
</table>

Energy Expenditure

| Total 24-hour (kcal)                | 1740 ± 71   | 1396 – 2088 |
| RMR (kcals/24h)                     | 1099 ± 36   | 919 – 1295  |
| RMR (kcal/kgFFM/24h)                | 26.0 ± 1.2  | 21.7 – 33.3 |
| RQ                                  | 0.88 ± 0.01 | 0.83 – 0.95 |
| Activity Monitor (kcal/24h)         | 641 ± 53    | 477 – 975   |

Body weight and body composition

Table 3.4 illustrates Pearson correlations between PYY, body composition, and energy balance parameters. No significant correlation was found between fasting PYY, 24-hour mean PYY or AUC and any measure of body composition.
### Table 3.4. Pearson correlation coefficients between PYY and energy balance parameters

<table>
<thead>
<tr>
<th></th>
<th>Fasting PYY (pg/ml)</th>
<th>Total AUC (pg/mlxday)</th>
<th>24-hour Mean PYY (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td>0.19</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.04</td>
<td>-0.10</td>
<td>-0.07</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>0.03</td>
<td>-0.10</td>
<td>-0.10</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>0.11</td>
<td>-0.04</td>
<td>-0.05</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Dietary Intake (3-day Diet Logs)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily Intake (kcal)</td>
<td>0.21</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>0.28</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.13</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.10</td>
<td>0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.04</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Energy Expenditure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 24-hour (kcal)</td>
<td>0.35</td>
<td>0.45</td>
<td>0.48</td>
</tr>
<tr>
<td>RMR (kcal/24hr)</td>
<td>0.52</td>
<td>0.69*</td>
<td>0.72*</td>
</tr>
<tr>
<td>RMR (kcal/kgFFM/24hr)</td>
<td>0.30</td>
<td>0.42</td>
<td>0.45</td>
</tr>
<tr>
<td>RQ</td>
<td>0.28</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td>Activity Monitor (kcal)</td>
<td>0.11</td>
<td>0.14</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*p < 0.05

### Dietary Intake from 3-day Diet Logs

Average daily energy intake obtained from 3-day diet logs is shown as total energy intake (1806 ± 124 kcal/day) and divided into macronutrient content per gram and percentage of carbohydrate, fat, protein and alcohol (Table 3.3). To determine the role of PYY in chronic energy intake PYY variables were correlated to variables obtained from the 3-day diet logs; however, no significant correlations were found (Table 3.4).

### Energy Expenditure

Table 3.4 also exemplifies significant correlations were found between RMR (kcal/24h) and 24-hour mean PYY (r = 0.71, p = 0.013) as well as total AUC (r = 0.69, p = 0.019). Figure 3.2B depicts the relationship between RMR and PYY 24-hour mean and
total AUC. Breakfast ($r =0.65$, $p = 0.03$), lunch ($r =0.69$, $p = 0.02$), and dinner ($r = 0.66$, $p = 0.03$) postprandial peaks were also positively correlated with RMR (kcal/24hr). No other significant correlations were found among any characteristics of PYY and body composition, chronic energy intake or EE.

Discussion

Diurnal Rhythm of PYY

To our knowledge, we are the first to report that PYY displays features of an energy-driven diurnal rhythm entrained by meal timing in healthy premenopausal women. Several groups have reported the 24-hour profile of PYY; however, these studies were in reference to pathophysiological conditions and obtained samples at time intervals where capturing meal responses may be compromised [6, 106-108]. Total energy and macronutrient content of the 24-hour sampling period were positively correlated to fasting PYY and all meal-related PYY parameters suggesting a meal-driven PYY response. In contrast to our data, Guo et al. [17] found no correlation between fasting PYY and 24-hour intake in obese and lean individuals. Additionally, postprandial peaks are all significantly elevated above fasting which demonstrates that the diurnal rhythm of PYY is driven by meal timing as well.

Fasting PYY Correlates

Positive correlations between fasting PYY and postprandial peaks as well as total AUC and 24-hour mean PYY provide evidence that higher fasting PYY concentrations are associated with a greater PYY response over the entire 24-hour period. This suggests that higher fasting PYY may be associated with enhanced satiety and subsequent weight regulation, and thus serve as a proxy indicator of PYY across the day. In support of our
findings a study by le Roux et al. [7] suggested that declines in fasting and postprandial PYY concentrations were associated with decreased satiety in obese compared to lean subjects whereas Batterham et al. [19] has shown that infusions of PYY lead to enhanced satiety in humans.

**PYY and Energy Intake**

Our data demonstrate that there was one PYY peak that is significantly higher than all other postprandial peaks even when there was no significant difference in meal energy content between lunch and dinner. Our finding that the meal rise is not significantly different between lunch and dinner thus indicates that the meal-induced PYY rise is dependent on the caloric content of the meal. Presumably, PYY was highest after lunch because the time between breakfast and lunch was relatively short (3h) and thus PYY concentrations remained elevated from the breakfast meal. This may suggest that allowing more time between meals permits more time for digestion/absorption and diminished PYY secretion. Additionally, our data best fit (highest R^2, lowest p-value) a cubic curvilinear function illustrating that PYY response is significantly altered across the day; highest at lunch and lowest after an overnight fast.

Several studies suggest more frequent meals across the day may allow for beneficial metabolic effects as well as greater 24-hour satiety [103-105]. Because the PYY response is meal driven more frequent meals across the day may allow for the maintenance of higher circulating PYY and perhaps, greater 24-hour satiation. Whether changing meal timing would affect peak PYY concentrations is unknown; however, we speculate that this would modify the 24-hour pattern we observed. Little is known about
the diurnal rhythm of PYY; therefore, it may be advantageous to explore other modulators of PYY beyond what we have investigated.

PYY is considered a satiety hormone among others that display similar meal-response patterns. Glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) also act in concert in response to nutrient ingestion to comprise the satiety response. GLP-1 is co-secreted with PYY from L cells in the gut, thus it is possible that these two hormones share a similar diurnal rhythm. It has been shown that PYY and GLP-1 concentrations both rise 5-30 minutes postprandially, likely due to vagal stimulation before nutrients reach the gut. Additionally, the effects of these peptides act as an “ileal brake [159].” GLP-1, like PYY, has been shown to be reduced in obesity [160]. However, GLP-1 has been shown to be reduced, whereas PYY has been shown to be elevated, in young anorexic females [161].

CCK is secreted from I cells and acts similarly to PYY and GLP-1. It rises 10-30 minutes postprandially and like PYY, responds more robustly to fat and protein ingestion more so than carbohydrate [162]. Additionally, the release of PYY has been demonstrated in dogs to be dependent upon CCK [96]. Lastly, unlike PYY, fasting CCK has been shown to be elevated in obese women and decreased in anorexia nervosa in comparison to lean control subjects [163].

**PYY and Energy Balance**

The role of PYY in energy balance is unclear as evidenced by several disparities in the current literature which may be the result of studying varying populations [5, 7, 9, 17]. Contrary to our finding, one study showed a negative correlation between fasting PYY and indices of EE like RMR (kcal/24h) and RQ in obese individuals in response to a
single meal [17]. However, our subjects were normal weight, sedentary women and our results reflect the complete 24-hour profile of PYY in response to three meals. This discrepancy may suggest a mechanistic disparity in pathophysiological conditions where the positive correlation seen in our study is lost in obesity where blunted fasting and circulating PYY occurs [7] or in energy deficiency or gastric-bypass patients where PYY is elevated [5, 9]. This correlation may exemplify a role for PYY in EE under normal physiological conditions where higher circulating PYY may be driving metabolism throughout the entire 24-hour period and/or vice versa. It is possible that greater circulating concentrations of PYY may be acting due to activation of hypothalamic receptors where PYY binds to the Y2R and inhibits NPY secretion and its orexigenic effects. Y2R binding also activates POMC neurons enhancing anorexigenic effects and increasing energy expenditure [114, 115]. To the contrary, higher resting metabolisms may be driving a greater secretion of PYY; however, a mechanism by which metabolism may stimulate PYY secretion has yet to be elucidated.

Though PYY correlated with dietary intake from the day of the 24-hour blood sampling, no correlations were found in relation to 3-day diet logs, suggesting that PYY plays a role in short-term satiety and meal cessation as opposed to chronic energy intake. Additionally, there were no correlations found between any PYY parameter and any measure of body composition and thus, PYY may not play a role in long term energy storage.

**Limitations**

This study was originally designed to measure physiological responses to meals occurring at known times of the day. Measures of satiety like visual analogue scales
were not utilized and thus this study can only speculate as to the effect of greater responses in PYY on satiety. Additionally, total PYY was assayed which includes both PYY$_{1-36}$ and PYY$_{3-36}$; however, when measuring 24-hour PYY it may be beneficial to capture both forms of PYY as both forms may be physiologically relevant [15].

Conclusions

PYY can be characterized as having a meal-driven diurnal rhythm as illustrated by significant correlations between PYY and numerous meal-related parameters exemplifying that meal timing as well as caloric load of a meal elicit postprandial responses contributing to the 24-hour profile. PYY’s role in EE is correlated to absolute resting metabolism; however, a defining role in this area needs further attention. Our data further support the hypothesis that PYY plays a significant role in energy balance as a satiety hormone and correlate of EE.
Chapter 4


Abstract

Peptide YY (PYY) and ghrelin (GHR) may modulate one another’s actions within the hypothalamus. Peripheral infusion of PYY in humans acutely suppresses circulating concentrations of GHR. Whether an association between PYY and GHR exists in the peripheral circulation of humans over 24h is unknown. The purpose of this study was to determine if circulating concentrations of PYY and GHR were significantly associated over 24h in humans. Participants (n=13) were normal weight, moderately active, women ages 18 – 24 y. Blood samples were obtained q10 min for 24h and assayed using RIA for total PYY and total GHR hourly from 0800–1000h and 2000–0800h and q20 min from 1000–2000h. Dietary intake during the 24h procedure was comprised of 55 % carbohydrates, 30 % fat, and 15 % protein (three meals and a snack). Statistical analyses included linear mixed-effects modeling to test whether PYY predicted GHR concentrations over 24 h. Participants weighed 57.0 ± 1.5 kg and had 26.1 ± 1.5 % body fat (15.0 ± 1.1 kg), 42.1 ± 1.1 kg fat free mass, a BMI of 21.3 ± 0.5 kg/m² and RMR of 1072 ± 28 kcal/24h. Visually, PYY and GHR exhibited an inverse association over nearly the entire 24 h period. Statistically, circulating concentrations of 24 h PYY predicted 24 h GHR (ghrelin = 1860.51 – 2.14*PYY; p = 0.04). Circulating concentrations of PYY are inversely associated with GHR over 24 h. These data provide evidence that PYY may contribute to the modulation of the secretion of GHR in normal weight, premenopausal women over a 24 h period and supports similar inferences from experimental studies in animals and humans.
**Introduction**

Studies using animal models [19, 164] and humans [3, 13, 20, 165, 166] support the roles of ghrelin and peptide YY (PYY) in the modulation of energy intake. Whereas PYY is one of several satiety hormones, ghrelin is the only hunger hormone that has been identified to date. It has been proposed that ghrelin and PYY oppose the actions of one another at the hypothalamus [22]. However, these hormones are secreted from cells along the gastrointestinal (GI) tract in the periphery and secretion is stimulated by caloric as well as macronutrient content of meals [2, 13, 20]. PYY may be involved in slowing GI motility and suppressing gastric acid secretion [23] whereas ghrelin stimulates those same actions [24]. One study infused ghrelin and demonstrated an attenuation of the effects of PYY on gastric acid secretion and motility [25]. Thus, ghrelin and PYY may oppose the actions of one another on digestion and absorption within the GI tract.

Although ghrelin and PYY may reciprocally inhibit the actions of one another on the GI tract, the mechanism through which ghrelin and PYY may modulate the actual secretory profiles of one another in the periphery is unknown [26]. Ghrelin decreases, whereas PYY increases post-prandially and thus, the reciprocal association may exclusively be a result of the incidental regulation by way of nutrient ingestion. However, evidence to support a regulatory association between ghrelin and PYY was demonstrated when infusion of PYY in the fasted state of obese and lean individuals suppressed pre- and postprandial circulating concentrations of ghrelin [8]. In light of this, it is attractive to hypothesize that ghrelin and PYY may be directly involved in modulating the secretion of one another in a negative feedback manner in the periphery. However, no study has examined whether an inverse association between ghrelin and PYY exists in the
peripheral circulation of healthy, normal weight humans over an entire 24h period. To that end, the current study sought to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation. We hypothesized that circulating concentrations of total PYY would be inversely associated with circulating concentrations of total ghrelin over 24h in normal weight, premenopausal women.

**Participants and Methods**

**Experimental Design and Participants**

We examined the 24 h profiles of total PYY and total ghrelin in 13 women ages 18 – 24 y. Non-smoking, non-exercising (< 1 h/wk exercise) women with a body weight (BW) of 48 – 73 kg, 15 – 30 % body fat and BMI between 18 – 25 kg/m² were included. Exclusion criteria included evidence of disordered eating or history of an eating disorder, loss/gain of BW (± 2.3 kg) in the past year, or use of hormonal contraceptives or medications that may alter metabolism. Participants completed an informed consent that was approved by the Biomedical Institutional Review Board of The Pennsylvania State University.

**Determination of Weight Maintenance Energy Requirements**

Participants were prescribed caloric intake to maintain BW estimated based 24 h energy expenditure (EE). This was determined from the sum of 24 h resting metabolic rate (RMR; kcal/24h) and EE above rest determined with the use of a research accelerometer (AM) worn over 7 days that measured the energy cost of all non-purposeful activity (triaxial RT3 accelerometer, Stayhealthy, Monrovia, CA). BW was recorded to the nearest 0.01 kg. RMR using indirect calorimetry and hydrostatic weighing
to obtain body composition were performed using previously published methods [20]. Participants were deemed weight stable if BW, obtained once daily in the morning, fluctuated by ± 1 kg over a period of 7 days.

24-hour Repeated Blood Sampling Protocol

Participants arrived fasted (8 – 12h) and abstinent from exercise or caffeine ingestion (24h) at the general clinical research center (GCRC) at 0730h on the day of testing. After insertion of an IV catheter into a forearm vein, blood samples were obtained q 10min for 24h (total = 488mL) while participants remained supine. Samples were allowed to clot at room temperature and spun in a centrifuge for 15min at 2500rpm. Serum samples were untreated, aliquoted to serum storage tubes and stored at -80ºC until analysis.

Dietary intake (total = 1452 ± 48 kcal) was comprised of 55 % carbohydrates, 30 % fat, and 15 % protein and consisted of 3 meals and a snack prepared for 0900 h (412 ± 28 kcal), 1200 h (472 ± 24 kcal), 1800 h (504 ± 0 kcal) and 2100 h (66 ± 4 kcal). Energy provided to participants was 85 % of their prescribed weight maintenance energy needs to account for reductions in EE due to inactivity associated with bed rest. Participants consumed all food provided within 30 min at each meal.

Radioimmunoassay Analysis

Serum samples were assayed for total ghrelin using the Linco Research RIA kit (St. Charles, MO). Assay sensitivity was 100 pg/mL. The intra-assay and inter-assay coefficients of variation for the high and low controls were 2.7 % and 16.7 % and 1.2 % and 14.7 %, respectively. Total PYY was assayed using an RIA (Millipore, Billerica, MA). PYY assay sensitivity was 10 pg/mL and the intra-assay and inter-assay
coefficients of variation were 2.9 % and 7.1 %, respectively. Samples were assayed hourly from 0800 – 1000 h and from 2000 – 0800 h and every 20 minutes from 1000 – 2000 h.

**Data and Statistical Analysis**

Fasting PYY and fasting ghrelin were designated as the concentrations at 0800h. Peaks were defined as the highest concentration measured 1 – 2 h before (ghrelin) or after (PYY) meal administration. Twenty-four h mean represented the average of all concentrations measured over 24 h. Total area under the curve (AUC) was calculated using the trapezoidal rule.

Linear mixed effects (random coefficients) models were fitted to participants’ responses to determine if PYY concentrations were associated with ghrelin concentrations over 24 h. A regression coefficient was considered significantly different from 0 if the p-value of the corresponding (t | z) test was ≤ 0.05. Data are reported as mean ± SEM. All analyses were performed using SPSS software (Version 18.0; Chicago, IL).

**Results**

Participants were non-exercising, normal weight (57.0 ± 1.5 kg) premenopausal women between the ages of 18 and 24 yr. Participants had 26.1 ± 1.5 % body fat (15.0 ± 1.1 kg), 42.1 ± 1.1 kg fat free mass and a BMI of 21.3 ± 0.5 kg/m². Average VO₂max was 37.8 ± 1.4 ml/kg/min. This value is between the 50th and 55th percentile for women ages 20 – 29 (ACSM Guidelines 8th Ed.) and likely consistent with a moderately active lifestyle. Average RMR was 1072 ± 28 kcal/24h and EE from AM was 658 ± 54 kcal.

Characteristics of ghrelin and PYY are presented in Table 4.1. Fasting ghrelin (1863 ± 132 pg/ml) and fasting PYY (68.7 ± 7.1 pg/ml), preprandial (ghrelin) and
postprandial (PYY) meal peaks and two indices of the 24 h profiles of ghrelin and PYY, AUC and 24 h mean, are presented for all participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ghrelin (Mean ± SEM)</th>
<th>PYY (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (pg/ml)</td>
<td>1862 ± 132</td>
<td>68.7 ± 7.1</td>
</tr>
<tr>
<td>Total AUC (pg/ml×24h)</td>
<td>37658 ± 1857</td>
<td>1932.8 ± 163.4</td>
</tr>
<tr>
<td>24h Mean (pg/ml)</td>
<td>1627 ± 79</td>
<td>85.3 ± 7.2</td>
</tr>
<tr>
<td>Breakfast Peak (pg/ml)</td>
<td>1862 ± 132</td>
<td>98.8 ± 9.9</td>
</tr>
<tr>
<td>Lunch Peak (pg/ml)</td>
<td>1701 ± 95</td>
<td>113.3 ± 10.0</td>
</tr>
<tr>
<td>Dinner Peak (pg/ml)</td>
<td>1960 ± 92</td>
<td>100.5 ± 8.4</td>
</tr>
<tr>
<td>Nocturnal Peak (pg/ml)</td>
<td>1906 ± 78</td>
<td>-------</td>
</tr>
<tr>
<td>Breakfast Nadir (pg/ml)</td>
<td>1418 ± 88</td>
<td>81.4 ± 8.9</td>
</tr>
<tr>
<td>Lunch Nadir (pg/ml)</td>
<td>1346 ± 75</td>
<td>77.2 ± 6.3</td>
</tr>
<tr>
<td>Dinner Nadir (pg/ml)</td>
<td>1448 ± 88</td>
<td>77.7 ± 8.1</td>
</tr>
<tr>
<td>Nocturnal Nadir (pg/ml)</td>
<td>-------</td>
<td>63.4 ± 5.9</td>
</tr>
<tr>
<td>Time of Breakfast Peak (h)</td>
<td>08:40 ± 0:10</td>
<td>10:40 ± 0:08</td>
</tr>
<tr>
<td>Time of Lunch Peak (h)</td>
<td>12:00 ± 0:05</td>
<td>13:40 ± 0:22</td>
</tr>
<tr>
<td>Time of Dinner Peak (h)</td>
<td>18:00 ± 0:03</td>
<td>18:40 ± 0:04</td>
</tr>
<tr>
<td>Time of Nocturnal Peak (h)</td>
<td>01:00 ± 0:12</td>
<td>-------</td>
</tr>
<tr>
<td>Time of Breakfast Nadir (h)</td>
<td>10:40 ± 0:09</td>
<td>11:40 ± 0:07</td>
</tr>
<tr>
<td>Time of Lunch Nadir (h)</td>
<td>14:00 ± 0:14</td>
<td>16:40 ± 0:19</td>
</tr>
<tr>
<td>Time of Dinner Nadir (h)</td>
<td>19:40 ± 0:12</td>
<td>20:00 ± 0:11</td>
</tr>
<tr>
<td>Time of Nocturnal Nadir (h)</td>
<td>-------</td>
<td>06:00 ± 01:30</td>
</tr>
</tbody>
</table>

Note: Ghrelin meal peaks are preprandial and ghrelin meal nadirs are postprandial. PYY meal nadirs are preprandial and PYY meal peaks are postprandial.

Figure 4.1 depicts the 24h circulating profiles of total ghrelin and total PYY.

Analysis of the data with a linear mixed effects model indicated that circulating total PYY was a statistically significant predictor of total ghrelin during the 24h period (ghrelin = 1860.51–2.14*PYY; p = 0.04) such that increases in circulating concentrations of PYY were associated with decreases in circulating concentrations of ghrelin.
Figure 4.1. Composite profile of circulating concentrations of (●) total PYY (pg/ml) and (□) total ghrelin (pg/ml) over 24 hours illustrating meal administration time points and results of the linear mixed modeling analysis. Data are expressed as mean ± SEM; p < 0.05
Discussion

We have previously demonstrated that the diurnal rhythms of ghrelin [13] and PYY [20] are driven by food-related events such as meal energy content and meal timing. These peptides are thus intimately involved in the modulation of energy intake and may play a role in both acute [13, 20] and chronic [34] energy balance. Non-food-related events such as the nocturnal rise in ghrelin, typically observed between 0100 – 0400h [13], also occur. Thus, it is important to characterize the 24h patterns of these hormones in the same study and test their relation to each other. To that end, our results support a role for PYY in the modulation of ghrelin in that circulating concentrations of total PYY were inversely associated with circulating concentrations of total ghrelin over an entire 24h period. This statistical finding is obvious upon visual inspection. For example, PYY concentrations were highest subsequent to the lunch meal after which ghrelin concentrations were lowest. Additionally, the nocturnal peak in ghrelin occurred at 0100h after a long rise, and this rise and peak are coincident with a prolonged decline in PYY. This inverse relation between PYY and ghrelin might reflect a reduced inhibition of ghrelin as PYY declines.

Previous studies have proposed that the underlying mechanism by which ghrelin and PYY modulate energy balance operates at the level of the hypothalamus where these hormones cross the blood-brain-barrier [109] and bind to receptors in the arcuate nucleus [22]. Few studies have examined the association between ghrelin and PYY in the peripheral circulation [8, 25]. One study demonstrated that fasting concentrations as well as the pre-prandial rise in ghrelin were suppressed 2h subsequent to PYY infusion in obese and lean individuals [8]. To date, no study has demonstrated an association
between these two gut peptides in the peripheral circulation over an entire 24h period in moderately active, normal weight, premenopausal women. Notably, the participants in our study were weight stable for at least six months prior to their entry into the study. During abnormal energy balance conditions, such as obesity or periods of weight loss, PYY and ghrelin may not be associated in the circulation in the same way and thus, more research is needed to determine if an “uncoupling” occurs between ghrelin and PYY with changes in BW. The present study may be a valuable reference to which results from studies involving abnormal states of energy balance can be compared.

It is important to note that factors other than PYY may also be modulating ghrelin. This is supported during times when no apparent inverse relation between PYY and ghrelin is evident. For example, ghrelin concentrations continue to rise to peak concentrations just subsequent to lunch when PYY concentrations remain stable within the hour prior and are beginning to increase. As well, ghrelin begins to decline subsequent to its nocturnal peak when PYY continues to decrease to its nadir at 0400h. Currently, it is unclear what factors modulate ghrelin secretion during times other than meal-related events such as the nocturnal rise. One study demonstrated that sleep may inhibit ghrelin [167] and other studies have associated sleep deprivation with elevated ghrelin [168]; however, the mechanism through which this occurs is unknown. The increase in ghrelin that leads to the nocturnal peak has been interpreted as a post dinner rebound that is then impacted by the onset of sleep which causes the gradual decline in ghrelin observed subsequent to the nocturnal peak [167]. It is also attractive to hypothesize that other factors, the secretion of which ghrelin is known to stimulate such as growth hormone or cortisol [169], may be involved in the feedback inhibition of
ghrelin, particularly during sleeping hours when the modulation of ghrelin by food intake is not a contributing factor.

A limitation of this study is that we measured total PYY and ghrelin and not the more biologically active forms i.e., PYY$_{3-36}$ and acylated ghrelin. However, it may be advantageous to capture both forms as studies have demonstrated that PYY$_{1-36}$ as well as PYY$_{3-36}$ inhibit energy intake and suppress subjective hunger ratings [15] whereas acylated ghrelin and des-acyl ghrelin both stimulate energy intake [170].

In conclusion, circulating concentrations of PYY were inversely associated with circulating concentrations of ghrelin over 24h in normal weight, premenopausal women during a period of weight stability. These data provide corroborative evidence of a reciprocal association between PYY and ghrelin over a 24h period in women. These findings may substantiate inferences from experimental studies in humans and animals suggesting that changes in PYY may be modulating the secretion of ghrelin.
Chapter 5

Hill BR, De Souza MJ, Wagstaff DA, and Williams NI. The impact of weight loss on the 24-hour profile of circulating peptide YY and its association to 24-hour ghrelin in normal weight premenopausal women.

Abstract

Peptide YY (PYY) and ghrelin exhibit a reciprocal association and antagonistic physiological effects in the peripheral circulation. Research has yet to clarify the effect of weight loss on the 24h profile of PYY or its association to 24h ghrelin. PURPOSE To determine if diet- and exercise-induced weight loss affects the 24h profile of PYY and its association with 24h ghrelin in normal weight, premenopausal women. METHODS Participants (n=13) were assessed at baseline (BL) and after a 3-month diet and exercise intervention (Post). Blood samples obtained q10 min for 24h were assayed for total PYY and total ghrelin q60 min from 0800–1000h and 2000–0800h and q20 min from 1000–2000h. The ghrelin/PYY ratio was used as an index of hormonal exposure. Statistical analyses included paired t-tests and linear mixed effects modeling. RESULTS Body weight (-1.85±0.67kg; p=0.02), and body fat (-2.53±0.83%; p=0.01) decreased from BL to post. Ghrelin AUC (5252±2177pg/ml/24hr; p=0.03), 24h mean (216±90pg/ml; p=0.03) and peak (300±134pg/ml; p=0.047) increased from BL to post. No change occurred in PYY AUC (88.2±163.7pg/ml; p=0.60), 24h mean (4.8±6.9pg/ml; p=0.50) or peak (3.6±6.4pg/ml; p=0.58). The 24h association between PYY and ghrelin at baseline (p=0.04) was weakened at post (p=0.14); however, the ghrelin/PYY lunch ratio increased (p=0.01) indicating the potential for ghrelin predominance over PYY in the circulation.

CONCLUSIONS PYY and ghrelin are reciprocally associated during a period of weight
stability, but not following weight loss. An “uncoupling” may have occurred, particularly at lunch, due to factors that modulate ghrelin in response to weight loss.

**Introduction**

The gut-brain axis is a complex and interwoven cascade of enteroendocrine hunger and satiety hormones signaling to and from the brain. It is believed that signaling occurs when hormones secreted from the gastrointestinal (GI) system in response to a meal enter the circulation and either cross the blood-brain-barrier to bind to receptors in the hypothalamic arcuate nucleus [109] and/or signal to that same hypothalamic region through receptors on vagal afferent neurons [153]. Several hormones are involved in the regulation of the hunger and satiety response to a meal; however, research suggests that two hormones in particular, ghrelin and peptide YY (PYY), may be involved not only in the modulation of energy intake, but in the modulation of one another, possibly through opposing actions in the hypothalamus [22] as well as within the peripheral circulation [8, 171].

Ghrelin was originally discovered as a growth hormone secretagogue [172]; however, its ability to stimulate energy intake was later discovered [173] and is the only known peripherally secreted orexigenic hormone. Ghrelin is elevated in the fasting state, peaks prior to meal initiation and decreases subsequent to food intake [10, 13]. In response to weight loss, ghrelin increases in both normal weight [12] and obese individuals [174] and is believed to play a role in both acute and chronic energy balance.

PYY is one of many satiety hormones that contribute to the modulation of acute energy balance. Its circulatory pattern is characterized by low fasting concentrations and peak circulating concentrations one to two hours postprandially. PYY has been shown to
be involved in the modulation of chronic energy balance in several elegantly performed studies in animals [3, 7, 8, 15, 19]. Peripheral injection of PYY into male Wistar rats has led to inhibition of food intake and reduced weight gain [19]. Peripheral infusion of PYY to concentrations similar to that observed in the postprandial state in non-obese individuals has elicited decreases in acute energy intake and suppressed subjective ratings of appetite at an ad libitum meal two hours subsequent to infusion [19]. In short, these studies suggest that PYY plays the role of a postprandial satiety hormone that influences acute energy balance in humans. However, the role that PYY plays in chronic energy balance in animals has not been demonstrated in humans as weight loss has resulted in increases [36], decreases [37, 38] and even no change [40] in circulating concentrations of PYY. One study demonstrated that the concentrations of fasting and postprandial PYY remained low in overweight and obese individuals during a year-long period of weight regain that was preceded by participation in a 10-week weight loss intervention [38]. Thus, it remains unclear as to how fluctuations in body weight will impact circulating PYY. To date, studies involving weight loss have observed resultant changes in either fasting or single-meal responses in PYY, but no study has determined the impact of chronic weight loss on the 24-hour profile of circulating PYY.

PYY and ghrelin modulate energy balance through integration within the hypothalamic arcuate nucleus [21]. Evidence to support this was provided by Riedeger et al. [22] in male Wistar rats where injection of PYY into arcuate explants inhibited the activation of ghrelin-sensitive neurons. However, ghrelin and PYY receptors are expressed on a number of tissues in the periphery that include the adrenal gland [71] and adipose tissue [144]. As well, it has been demonstrated that ghrelin and PYY oppose the
actions of one another on the GI tract: PYY slows GI motility and suppresses gastric acid secretion [23], whereas ghrelin stimulates those same actions [24]. Chelikani et al. [25] demonstrated that intravenous infusion of ghrelin attenuated the effects of PYY infusion on gastric acid secretion and GI motility. Therefore, by focusing on hypothalamic regulation of these hormones we may limit our understanding of their reciprocal actions in the periphery.

Studies that have demonstrated an association between ghrelin and PYY in the circulation have mainly demonstrated this association following one meal [8, 25]. We have previously reported that the 24-hour profiles of circulating ghrelin and PYY are inversely associated over an entire 24-hours during a period of energy balance and weight stability [171]. Additionally, we previously reported that the 24-hour profile of ghrelin is elevated subsequent to diet- and exercise-induced weight loss in normal weight, premenopausal women. However, no study has determined if the association between the 24-hour profiles of ghrelin and PYY assessed during a period of weight stability remains subsequent to diet- and exercise-induced weight loss. Thus, the purpose of this study was two-fold: 1) to determine if diet- and exercise-induced weight loss would alter the 24-hour profile of total circulating PYY and 2) to determine if the association between the 24-hour circulating concentrations of PYY and ghrelin observed during a period of weight stability would be altered subsequent to a period of diet- and exercise-induced weight loss in normal weight, premenopausal women. We hypothesized that 1) the 24-hour profile of total circulating PYY would decrease subsequent to diet- and exercise-induced weight loss; 2) the 24-hour profile of total circulating ghrelin would increase subsequent to diet- and exercise-induced weight loss; and 3) the inverse association
between ghrelin and PYY observed at baseline will remain significant subsequent to diet- and exercise-induced weight loss in normal weight, premenopausal women.

Participants and Methods

Experimental Design and Participants

This study was part of a larger, prospective study designed to assess changes in endocrine and reproductive function in response to a 3-month, controlled feeding and exercise intervention. The intervention was implemented in sedentary women, who were between the ages of 18 and 30, to emulate the exercise and restrictive eating patterns in which many young women engage. During a four-week baseline period, participants were randomly assigned to an experimental group: a control group that did not exercise and consumed an amount of calories estimated to maintain body weight; a control group that exercised, but received extra calories as food to remain in energy balance; or one of four energy deficit groups that exercised and were prescribed reduced energy intake. Each group was defined by a particular prescription for the quantity of calories provided as food and the quantity of calories expended as exercise. Following screening and the baseline monitoring period, participants completed the 3 month diet and exercise intervention.

Participants included in the present study were normally active, normal weight women (n = 13), ages 18 – 24 years, who completed anthropometric, energy balance, and 24-hour repeated blood sampling procedures at baseline and following their completion of the intervention (post). All participants included in the present study had been randomly assigned to one of the four energy deficit groups who were prescribed and provided reductions in food calories and exercised in order to achieve a negative energy
balance ranging from – 30 % to – 60 % when compared with baseline energy needs. On average, the thirteen participants achieved a negative energy balance of - 29.6 ± 9.6 % less than their baseline energy needs. Because we previously reported in weight stable women that no change occurred in the concentrations of total circulating ghrelin during a 24-hour period in the 3-month parent study, a weight stable control group was not included in the present study.

**Screening**

Participants were non-smoking, healthy, non-exercising (< 1 hour/week purposeful exercise) women, who were between the ages of 18 and 30 years and had a BMI between 18 and 25 kg/m². Exclusion criteria included any evidence of disordered eating or history of an eating disorder, loss/gain of a significant amount of weight (± 2.3 kg) in the past year, or use of hormonal contraceptives or medication that may alter metabolic hormones. Each participant signed an informed consent letter approved by the Biomedical Institutional Review Board of The Pennsylvania State University.

Participants completed questionnaires to provide information regarding demographics, medical history, menstrual history, physical activity and eating attitudes. A fasting blood sample was obtained by a General Clinical Research Center (GCRC) nurse between 0600 and 1000 hours for analysis of a complete blood count and basic chemistry panel and to rule out abnormal pituitary function or metabolic diseases. Each participant’s psychological stability and the absence/risk of eating disorders were established in an interview conducted by a clinical psychologist. Participants met with a GCRC registered dietician to ensure the absence of aberrant dietary habits and suitability for a controlled feeding study.
Body Composition and Aerobic Fitness

Body weight was measured wearing shorts and a tee-shirt (without shoes) and recorded to the nearest 0.1 kg. Hydrostatic weighing after correcting for residual lung volume was utilized to measure body density which was used to calculate body composition using the Brozek equation [157]. Maximal aerobic capacity (ml/kg/min) was determined using indirect calorimetry at baseline and post according to previously published methods [34].

Energy Balance Parameters

Resting Metabolic Rate

Resting metabolic rate (RMR) was measured using a ventilated hood system between 0600-1000 hours following an overnight fast. Participants lay in the supine position for 20 – 30 minutes to acclimate to the room temperature and testing procedures; the hood was placed over the participant’s head for 30 minutes. Expired air was measured every minute for carbon dioxide and oxygen concentration using a carbon dioxide analyzer (URAS4, Hartmann & Braun, Frankfurt, Germany) and a paramagnetic oxygen analyzer (Magnos 4G, Hartmann & Braun). The values for minutes in which steady state was achieved were averaged to calculate RMR (kcal/day), determined using the Weir equation [158], and RQ.

Physical Activity Expenditure

To assess the energy cost of all physical activity energy expenditure (kcal) above resting, participants wore a tri-axial activity monitor (AM) (RT3 accelerometer, Stayhealthy, Monrovia, CA) 24 hours/day for a 7-day period. The AM, which was worn on the left hip, was not worn during showering/bathing. Participants recorded AM logs
which identified all the activities and periods of time when the monitor was not worn (i.e., sleeping and showering). No participants engaged in regular exercise at baseline and therefore, did not accumulate energy expenditure from exercise. Thus, to determine total 24-hour energy expenditure (kcal/24hr) at baseline, RMR and the average daily energy expenditure from AM were summed.

**Determination of Baseline Energy Needs**

Caloric intake required to maintain weight for each participant (baseline energy needs) was determined based on the calculation of 24-hour energy expenditure described above. During the baseline period, GCRC metabolic kitchen staff prepared and provided study participants with a prescribed diet for a 7-day calibration period. During this period, participants were weighed daily and ± 100 kcal adjustments were made if a participant’s body weight fluctuated by more than ± 1 kg. The 7-day diet, which was comprised of 55% carbohydrates, 30% fat, and 15% protein, provided the total amount of calories each participant required to maintain her baseline weight (1800 ± 64 kcal).

**Dietary Intake and Exercise Training During the Intervention**

After the baseline calibration period, participants were provided fewer calories and began exercise training supervised by research staff. The diet was comprised of 55% carbohydrate, 30% fat, and 15% protein [34]. All study meals were prepared and provided by registered dieticians in the GCRC metabolic kitchen. Participants were instructed to eat all of the food and only the food provided to them. Any food that was not eaten was re-weighed and the calorie amount was recorded for later subtraction from the prescribed intake total. Food eaten, but not prescribed by the study was highly discouraged. Any food consumed outside of that which the study prescribed was recorded
on a log sheet. Calories and macronutrient content were calculated using Nutritionist Pro (First Data Bank, Indianapolis, IN).

Participants performed aerobic exercise five times per week at 70 – 80% of maximum heart rate as determined from tests of maximal aerobic capacity. The total amount of calories expended during each exercise session was measured using the OwnCal feature on the Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland).

Daily and weekly averages of 24-hour energy intake were closely monitored throughout the study to ensure participant compliance. As well, body weight and 24-hour energy expenditure were repeatedly measured during the intervention. Minor adjustments were made in caloric intake and exercise energy expenditure to meet the prescribed energy deficit. The energy deficit created through diet and exercise averaged - 29.9 ± 9.6 % less than baseline energy needs.

**Twenty-four-hour Repeated Blood Sampling**

All participants underwent the 24-hour assessment in the follicular phase (days 2 – 7) at least one week after the baseline calibration period and subsequent to the 3-month diet and exercise intervention. Participants were instructed to abstain from exercise or caffeine ingestion 24 hours prior to the testing day and to fast as of 2000 hours the night prior. Participants arrived at the GCRC at 0730 hours on the day of testing. For the 24-hour blood draw, they remained in a supine position with their upper body slightly elevated and an intravenous catheter was inserted in a forearm vein. Blood samples were obtained every 10 minutes for 24 hours (total = 144 samples). 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 2500rpm. Serum aliquots were transferred to storage tubes and stored in a - 80º Celsius freezer until analysis.

All meals during the 24-hour blood 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 % carbohydrates, 30 % fat, and 15 % protein and consisted of three meals and a snack prepared at 0900 hours (breakfast), 1200 hours (lunch), 1800 hours (dinner) and 2100 hours (snack). Dinner consisted of 504 ± 0.4 kcal and the remainder of kcal provided was distributed between breakfast (412 ± 28 kcal), lunch (472 ± 24 kcal) and the snack (64 ± 3 kcal). Participants knew when meals were to be served and were required to eat all/only the food provided. To account for reductions in energy expenditure due to inactivity associated with bed rest, the caloric prescription for the 24-hour blood sampling period provided participants with 85 % of their calculated baseline energy needs. All meals provided at the baseline and post 24-hour repeated blood sampling analyses were identical and reflective of what participants typically consumed. Meals consisted of foods like English muffins, orange juice, turkey lunchmeat sandwiches, grapes, and pork stir-fry.

Total Ghrelin

Total ghrelin was measured in duplicate in each serum sample from the 24-hour repeated blood draws. Specifically, total ghrelin was measured 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- and inter-assay coefficients of variation for the high control
were 2.7 % and 16.7 %, respectively; the intra- and inter-assay coefficients of variation for the low control were 1.2 % and 14.7 %, respectively. All samples from a given participant were analyzed within the same assay.

**Total PYY**

Total PYY was measured in duplicate in each serum sample from the 24-hour repeated blood draw. Specifically, total PYY was measured hourly from 0800 to 1000 hours, every 20 minutes from 1000 to 2000 hours, and hourly from 2000 to 0800 hours using a radioimmunoassay (Millipore, Billerica, MA). The sensitivity of the assay was 10 pg/ml and the intra- and inter-assay coefficients of variation were 2.9 and 7.1 %, respectively. All samples from a given participant were analyzed within the same assay.

**Data Analysis**

**Ghrelin and PYY Analysis**

Fasting ghrelin concentrations represented the smallest of the ghrelin concentrations measured between the hours of 0400 and 0700 hours to avoid any influence of meals or meal timing. Fasting PYY concentrations were denoted as the smallest PYY concentration observed between the first morning blood draw (typically 0800 hours) and 0900 hours just prior to the breakfast meal administration. The 24-hour means for both ghrelin and PYY were calculated as the average of the 44 concentrations (pg/ml) measured during the 24-hour analysis. Area under the curve (AUC) was calculated using the trapezoidal rule. Meal peaks were defined as the largest concentration (pg/ml) that were measured prior (ghrelin) or subsequent to (PYY) meal administration. Meal nadirs were defined as the smallest concentration (pg/ml) that were measured prior (PYY) or subsequent to (ghrelin) meal administration.
To determine when changes occurred in the association between ghrelin and PYY across 24-hours and from baseline to post in response to the intervention, we used the ghrelin-to-PYY ratio (ghrelin/PYY) at each time point (44 points per participant) as an index of the relative exposure of the body to each peptide. We reasoned that if, for example, the ratio increases across the day, or over time with an intervention, it could be interpreted in a number of ways: 1) increases in the ghrelin concentration occurred simultaneously with increases in the PYY concentration that were of a lesser magnitude than the increases observed in ghrelin, 2) increases in the ghrelin concentration occurred simultaneously with decreases in the PYY concentration, 3) for a given PYY concentration, the ghrelin concentration was increasing, or 4) for a given ghrelin concentration, the PYY concentration was decreasing. Corresponding, but opposite explanations could be used for decreases in the ratio. Increases in the ghrelin/PYY ratio may thus suggest that the influence of ghrelin on physiological processes may be greater than that of PYY and the opposite may hold if the ratio were to decrease.

We also sought to demonstrate at which specific events (i.e., meal related or nocturnal events) the exposure to ghrelin and PYY changed. To accomplish this, we calculated the mean of the 44, 24-hour ghrelin/PYY ratios (24-hour Ghrelin/PYY ratio) as well as the mean ghrelin/PYY ratio for all meals (breakfast, lunch and dinner) and the nocturnal event for each of the thirteen participants at baseline and at post. The mean of each meal or nocturnal event ghrelin/PYY ratio was calculated by averaging the ratios corresponding to the time the meal was served or lights out (nocturnal event) to the time point prior to the time that marked the start of the next event. For example, the breakfast mean ghrelin/PYY ratio was calculated by averaging the ratios corresponding to the start
time of 0800 hours to an end time of 1140 hours (prior to the lunch meal). The lunch mean ghrelin/PYY ratio was calculated by averaging the ratios corresponding to a start time of 1200 hours to an end time of 1740 hours (prior to the dinner meal). The dinner mean ghrelin/PYY ratio was calculated by averaging the ratios corresponding to a start time of 1800 hours to an end time of 2200 hours (prior to lights out). Ratios corresponding to the snack were included within the dinner mean ghrelin/PYY ratio calculation because there was no statistically significant increase or decrease in the concentrations of PYY or ghrelin, respectively, in response to the snack meal from 2100 hours to 2300 hours (p > 0.05). Lastly, the nocturnal mean ghrelin/PYY ratio was calculated by averaging the ratios corresponding to a start time of 2300 hours (lights out) to an end time of 0800 hours (the last data point collected the following morning).

**Statistical Analysis**

Preliminary analyses were used to identify outliers, missing data and other data problems. Only extreme data points were considered for exclusion. Casewise diagnostics were run using simple linear regression pairwise residual analysis on the 44 time points of the 24-hour profiles of ghrelin and PYY. Outliers that were detected encompassed both ghrelin and PYY concentrations at the denoted time point and were removed from further analysis. Eight (four baseline and four post) of the 572 values (13 participants with 44 values) met the criterion used to identify an outlying value and were excluded from statistical analyses. Paired t-tests were used to determine if statistically significant mean differences were observed from baseline to post with respect to individual ghrelin and PYY parameters (i.e., AUC, 24-hour means, pre- and postprandial peaks, etc.) as well as the mean 24-hour, meal-related and nocturnal ghrelin/PYY ratios.
A linear mixed effects (random coefficients) model was fitted to participants’ responses to determine if PYY concentrations were linearly related with ghrelin concentrations over the 24-hour period at baseline and at post. Ghrelin was considered the dependent variable and was regressed on the independent variable, PYY, which was entered into the model as both a fixed and random effect. Time was entered at a repeated measure. The baseline value was treated as the first measurement. The random coefficients model allows regression coefficients to vary across individuals. Consequently, each participant can have her own regression model. Additionally, estimation of the regression coefficients is relatively straightforward even when there are missing outcome values [175]. For all models, a regression coefficient was considered to be statistically different from 0 if the p-value of the corresponding test statistic was ≤ 0.05. Data are reported as mean ± SEM. All analyses were performed using SPSS software (Version 18.0; Chicago, IL).

Results

Participants

Participants’ demographic characteristics at baseline are presented in Table 5.1. The thirteen participants were non-exercising (< 1 hour purposeful exercise/week), normal weight women ranging in age from 18 to 24 years. Participants had remained weight stable (± 2.3 kg) within the six months prior to entry into the study. Average $\text{VO}_{2\text{max}}$ at baseline was 37.8 ± 1.4 ml/kg/min. This value is between the 50th and 55th percentile for women ages 20 – 29 (ACSM Guidelines 8th Ed.) and likely consistent with a moderately active lifestyle. Ten (77 %) participants were Caucasian, two (15 %) were African-American, and one was Hispanic (8 %).
Table 5.1. Subject demographics at baseline and post (n=13)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (Mean ± SEM)</th>
<th>Range</th>
<th>Post (Mean ± SEM)</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>20 ± 0.5</td>
<td>18 – 24</td>
<td>20 ± 0.5</td>
<td>18 – 24</td>
<td>n/a</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.7 ± 1.4</td>
<td>154.3 – 170.2</td>
<td>163.7 ± 1.4</td>
<td>154.3 – 170.2</td>
<td>n/a</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>57.0 ± 1.5</td>
<td>51.1 – 66.5</td>
<td>55.2 ± 1.4</td>
<td>48.0 – 65.5</td>
<td>0.02*</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>21.3 ± 0.5</td>
<td>18.8 – 23.9</td>
<td>20.6 ± 0.5</td>
<td>18.0 – 23.6</td>
<td>0.02*</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>26.1 ± 1.5</td>
<td>19.0 – 36.8</td>
<td>23.5 ± 1.3</td>
<td>18.6 – 34.5</td>
<td>0.01*</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>15.0 ± 1.1</td>
<td>9.8 – 22.2</td>
<td>13.1 ± 1.0</td>
<td>9.2 – 21.2</td>
<td>0.01*</td>
</tr>
<tr>
<td>Fat Free Mass (kg)</td>
<td>42.1 ± 1.1</td>
<td>37.2 – 49.6</td>
<td>42.1 ± 1.0</td>
<td>36.0 – 47.0</td>
<td>0.97</td>
</tr>
<tr>
<td>VO₂ max (kcal/kg/min)</td>
<td>37.8 ± 1.4</td>
<td>29.8 – 42.7</td>
<td>43.5 ± 1.6</td>
<td>36.4 – 52.9</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*p < 0.05
Effect of the Intervention on Body Composition and Aerobic Fitness

Table 5.1 also displays the change from baseline to post in body composition and aerobic fitness. There was a statistically significant decrease in body weight (\(-1.85 \pm 0.67\) kg), BMI (\(-0.69 \pm 0.24\) kg/m\(^2\)) and body fat (\(-2.53 \pm 0.83\) %) from baseline to post. However, the change in fat free mass was not statistically significant (0.02 \pm 0.46 kg). Finally, there was a statistically significant increase in the participants’ aerobic fitness as measure by VO\(_{2}\)max (6.98 \pm 1.63 ml/kg/min) from baseline to post.

Ghrelin

Figure 5.1A depicts the 24-hour profile of circulating total ghrelin at baseline and post. There was a statistically significant increase in the circulating concentrations of ghrelin during the study period. Table 5.2 demonstrates the change from baseline to post in several ways that are often used to describe the concentrations of ghrelin in the body. Significant increases occurred from baseline to post in fasting ghrelin, total AUC, 24-hour mean ghrelin and the lunch, dinner and nocturnal peaks of ghrelin. The change from baseline to post in the ghrelin preprandial breakfast peak or the ghrelin postprandial breakfast, lunch and dinner nadirs was not statistically significant.
Figure 5.1. **A.** 24-hour profiles of total circulating ghrelin (pg/ml) at baseline and post. **B.** 24-hour Profiles of total circulating PYY (pg/ml) at baseline and post. Data are reported as mean ± SEM.
Table 5.2. Baseline and post characteristics of ghrelin and PYY

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ghrelin (Mean ± SEM)</th>
<th>PYY (Mean ± SEM)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (pg/ml)</td>
<td>1410 ± 72</td>
<td>1710 ± 92</td>
<td>0.005*</td>
<td>69.4 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>68.9 ± 6.7</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AUC (pg/mlx24h)</td>
<td>37658 ± 1857</td>
<td>42911 ± 2289</td>
<td>0.03*</td>
<td>1935.1 ± 162.7</td>
</tr>
<tr>
<td></td>
<td>1846.8 ± 161.3</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Hr Mean (pg/ml)</td>
<td>1627 ± 79</td>
<td>1844 ± 100</td>
<td>0.03*</td>
<td>85.3 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>80.5 ± 7.2</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast Peak (pg/ml)</td>
<td>1862 ± 132</td>
<td>1879 ± 136</td>
<td>0.85</td>
<td>98.7 ± 10.0</td>
</tr>
<tr>
<td></td>
<td>96.3 ± 10.4</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch Peak (pg/ml)</td>
<td>1701 ± 95</td>
<td>1951 ± 105</td>
<td>0.02*</td>
<td>113.4 ± 10.0</td>
</tr>
<tr>
<td></td>
<td>109.8 ± 9.7</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner Peak (pg/ml)</td>
<td>1960 ± 92</td>
<td>2260 ± 132</td>
<td>0.047*</td>
<td>100.5 ± 8.4</td>
</tr>
<tr>
<td></td>
<td>92.1 ± 8.0</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal Peak (pg/ml)</td>
<td>1906 ± 78</td>
<td>2222 ± 125</td>
<td>0.03*</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>-----</td>
<td>-----</td>
<td></td>
<td>-----</td>
</tr>
<tr>
<td>Breakfast Nadir (pg/ml)</td>
<td>1419 ± 88</td>
<td>1355 ± 111</td>
<td>0.41</td>
<td>81.4 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>76.5 ± 9.0</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch Nadir (pg/ml)</td>
<td>1346 ± 75</td>
<td>1455 ± 126</td>
<td>0.27</td>
<td>77.2 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>68.8 ± 8.4</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner Nadir (pg/ml)</td>
<td>1448 ± 88</td>
<td>1589 ± 90</td>
<td>0.21</td>
<td>77.7 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>70.2 ± 6.6</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal Nadir (pg/ml)</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>63.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>-----</td>
<td>-----</td>
<td>0.08</td>
<td>62.1 ± 5.4</td>
</tr>
</tbody>
</table>

Note: PYY meal nadirs are preprandial and meal peaks are post-prandial whereas ghrelin meal peaks are pre-prandial and meal nadirs are postprandial. Nocturnal peak is only analyzed in ghrelin. Nocturnal nadir is only analyzed in PYY.

*p<0.05
PYY

Figure 5.1B depicts the 24-hour profile of circulating total PYY at baseline and post. The change in circulating total PYY from baseline to post was not statistically significant. Table 5.2 demonstrates the change from baseline to post in several ways that are often used to describe the concentrations of PYY in the body. No statistically significant change was observed in fasting PYY, total AUC, 24-hour mean PYY, the preprandial breakfast lunch, dinner and nocturnal PYY nadirs or the breakfast lunch, and dinner postprandial PYY peaks.

The Association between Ghrelin and PYY

Figure 5.2 depicts the 24-hour profiles of total ghrelin and total PYY at baseline (A) and post (B). We have previously reported the association at baseline between the 24-hour profiles of total circulating ghrelin and total circulating PYY [171]. The results of the linear mixed effects model demonstrated that total circulating PYY was a statistically significant predictor of total circulating ghrelin over 24 hours at baseline during a period of energy balance and weight stability (Ghrelin (pg/ml) = 1860.51 – 2.14*PYY; p = 0.04). Subsequent to the 3-month diet and exercise intervention that elicited a significant decrease in body weight of - 1.85 ± 0.7 kg, the reciprocal association between PYY and ghrelin was no longer detected (Ghrelin (pg/ml) = 1811.3 + 1.8*PYY; p = 0.14).

As stated, we used the ratio of ghrelin to PYY across a 24-hour period at baseline and at post as an index of hormonal exposure. Figure 5.2C displays the ghrelin/PYY ratios at each time point in the 24-hour period at baseline and at post. Predominance in circulating concentrations of ghrelin over PYY is exhibited by an increase in the ghrelin/PYY ratios at post. A statistically significant increase in the lunch mean
ghrelin/PYY ratio was found. There were trends toward statistically significant increases in the mean dinner, nocturnal event and 24-hour ghrelin/PYY ratios from baseline to post (Table 5.3).
Figure 5.2. A. Baseline 24-hour profiles of total PYY (pg/ml) and total ghrelin (pg/ml) illustrating the results of the linear mixed effects modeling: Ghrelin (pg/ml) = 1860.51 – 2.14*PYY; p = 0.04. Adapted from Hill et al. 2012 with permission (See appendix for license agreement). B. Post 24-hour profiles of total PYY (pg/ml) and total ghrelin (pg/ml) illustrating the results of linear mixed effects modeling: Ghrelin (pg/ml) = 1811.3 + 1.8*PYY; p = 0.14. C. 24-hour profile of the Ghrelin/PYY ratio at baseline and post (note: solid lines above the profiles denote the concentrations included in the calculation of the meal or nocturnal ghrelin/PYY ratio). Data are reported as mean ± SEM. *p < 0.05.
Table 5.3. Ghrelin/PYY ratio variables at baseline and post

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (Mean ± SEM)</th>
<th>Post (Mean ± SEM)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast Mean</td>
<td>23.3 ± 2.9</td>
<td>23.2 ± 2.2</td>
<td>0.96</td>
</tr>
<tr>
<td>Lunch Mean</td>
<td>20.1 ± 1.8</td>
<td>24.1 ± 1.6</td>
<td>0.01*</td>
</tr>
<tr>
<td>Dinner Mean</td>
<td>22.0 ± 2.4</td>
<td>25.5 ± 1.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Nocturnal Mean</td>
<td>26.4 ± 2.7</td>
<td>31.0 ± 2.1</td>
<td>0.08</td>
</tr>
<tr>
<td>24-hour Mean</td>
<td>22.2 ± 2.2</td>
<td>25.5 ± 1.7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*p < 0.05

Discussion

To our knowledge, this is the first report of changes in the 24-hour profile of circulating concentrations of PYY in response to modest diet- and exercise-induced weight loss in normal-weight, premenopausal women. As expected, the 24-hour profile of circulating ghrelin increased in response to diet- and exercise-induced weight loss. However, contrary to our hypothesis, no statistically significant change from baseline to post was observed in any aspect of the 24-hour profile of circulating PYY. This is the first account of changes in the association between circulating concentrations of ghrelin and PYY over 24 hours subsequent to diet- and exercise-induced weight loss in humans. Our findings demonstrate that the reciprocal association between PYY and ghrelin observed during a period of weight stability at baseline was weakened at post, subsequent to diet- and exercise-induced weight loss. The ghrelin/PYY ratio analyses revealed that the loss of the association between ghrelin and PYY across the day occurred mostly at lunch as exhibited by statistically significant increases in the lunch mean ghrelin/PYY ratio. This change suggests that after modest weight loss, the lunch time might be a time of day where the physiological drive to eat may predominate relative to other times of the day, but more research is needed to support this notion.
Several studies have reported varying results of fasting or changes in single meal-related responses in postprandial PYY subsequent to weight loss [36-38, 40]. In the current study, we demonstrated that no change occurred in the entire 24-hour profile of total circulating PYY in response to diet- and exercise-induced weight loss in previously untrained normal weight, premenopausal women. Several conclusions might be drawn due to varying results with regard to how circulating PYY may change in response to changes in body weight. Circulating PYY may simply not respond to changes in body weight comparable to what is observed with ghrelin where weight loss elicits increases in circulating ghrelin [12]. Moreover, secretion of PYY may be less sensitive than ghrelin to changes in body weight. Specifically, greater changes in body weight may be required to elicit alterations in the circulating concentration of PYY. The average decrease in body weight observed here, in normal weight women where no change in PYY was observed, was small (i.e., 1.8 ± 0.7 kg body weight). Scheid et al. [40] demonstrated no change in fasting PYY in response to 3.2 ± 0.8 kg weight loss in normal weight, premenopausal women. However, Sumithran et al. [38] observed a 13.5 ± 0.5 kg decrease in body weight concomitant with highly significant decreases in fasting and postprandial PYY that persisted through an entire year even during a period of weight re-gain. Consequently, it may be that there exists a threshold with respect to a loss of body weight, above which significant decreases in PYY are observed. On the contrary, PYY may decrease in response to greater losses in body weight, but may respond in a more favorable manner (i.e., PYY may increase) in response to favorable changes in body composition (i.e. body fat loss and/or increased lean body mass) where no concomitant loss of body weight occurs. A study conducted by Jones et al. [36] with overweight
adolescents and reported that fasting concentrations of total PYY increased in response to
an eight-month aerobic exercise intervention that resulted in significant body fat loss, but
not body weight loss. Because this study was conducted with adolescents, its findings
may not hold in an adult population. Future studies may focus on whether a dose-
response relationship exists between changes in body weight and changes in the profile of
circulating PYY to determine whether an association exists between chronic body weight
regulation and PYY.

There are few studies similar to the present study that have examined the
association between ghrelin and PYY in the peripheral circulation [8, 25]. Batterham et
al. [8] demonstrated that fasting concentrations of ghrelin decreased significantly and that
the pre-prandial rise in ghrelin was suppressed two hours subsequent to infusion of PYY
in obese and lean men and women. Although, it is noteworthy that these infusions
resulted in physiologically relevant doses of the PYY, infusions were only performed for
90 minutes and measured responses in ghrelin to a single meal. Additionally, Feinle-
Bisset et al. [92] showed that infusion of intraduodenal fat, a macronutrient known to
stimulate PYY secretion [90], also suppressed circulating concentrations of ghrelin in
young, healthy men.

The current study provides an extension of prior studies by furthering the
knowledge of the association between ghrelin and PYY over an entire 24-hour period and
taking into account fluctuations in the circulating concentrations of these hormones
during three meals, a snack and the nocturnal period. We have previously demonstrated
that PYY and ghrelin, both of which play a role in acute and chronic energy balance, may
be involved in the modulation of one another in a feedback mechanistic manner in the
peripheral circulation over an entire 24-hour period [171]. Our present findings demonstrate that the inverse association between PYY and ghrelin was weakened by a 3-month period of diet- and exercise-induced weight loss when circulating concentrations of ghrelin increased and no change was observed in circulating PYY. It is notable that increases in ghrelin were observed despite administration of exactly the same calorie and macronutrient content of meals during the 24-hour analyses. Significant increases in ghrelin concentrations from baseline to post were roughly 100 pg/ml to 325 pg/ml. These increases are similar to rises in ghrelin observed from meal nadirs and peaks observed in our data in a single day and thus, likely represent a physiologically relevant increase in ghrelin. Increases in circulating ghrelin with no concomitant change in PYY may be a physiological adaptation to weight loss and may be promoting weight regain; however, the mechanism by which ghrelin increases in response to weight loss is currently unknown.

Our data demonstrated that the association between ghrelin and PYY observed at baseline was weakened at post. Loss of the association between ghrelin and PYY may be a result of a counter-regulatory mechanism responding to a decrease in body weight by uncoupling the association between ghrelin and PYY and allowing for increases in ghrelin to occur to signal to the body to regain weight. Moreover, there may be events in the profiles of circulating ghrelin and PYY during which other factors that modulate ghrelin and/or PYY have influenced their appearance in the circulation and resulted in the uncoupling observed herein. To that end, our data demonstrated that a significant predominance of ghrelin exposure occurred at lunch, i.e., significant increases in lunch mean ghrelin/PYY ratio, from baseline to post. Additionally, ghrelin meal peaks
increased in magnitude across 24 hours in the post profile when compared to baseline. For example, the preprandial rises in ghrelin were greater prior to lunch than breakfast, greater prior to dinner than lunch or breakfast and greater prior to the nocturnal peak than any of the three meal peaks. Thus, the increases observed in ghrelin, particularly at lunch, may be altering the 24-hour profile of ghrelin such that greater increases in ghrelin across the day predominate and overcome the potential negative feedback of PYY on ghrelin. Consequently, ghrelin predominance may stimulate an increase in energy intake on that day or during subsequent days, ultimately resulting in the potential for weight regain.

Additionally, there was an unexpected increase in PYY between 1600 and 1800 hours in the post profile of circulating PYY. These hours correspond to the time between the lunch and dinner meals when no meal has been served. Although, the event or events that cause this rise are not known, it is possible that other factors that may be involved in the modulation of PYY such as vagal nerve stimulation where increases in circulating concentrations of PYY are observed within the first fifteen minutes of food consumption and prior to nutrients reaching the region of the gut from which PYY is secreted [100]. The observed increase in PYY did not occur with any corresponding decrease in ghrelin. Thus, an uncoupling in response to weight loss may allow for other factors to modulate changes in the circulating profiles of PYY and ghrelin.

Strengths of this study are several-fold. First, a calibration period was incorporated at least one week prior to the 24-hour blood sampling when participants were provided an eucaloric diet to maintain weight stability. Consequently, participants were in a state of energy balance during the testing periods of the 24-hour analyses which also provided a stable comparison for the post analyses when individuals had lost weight.
Participants were provided the same dietary intake on 24-hour blood sampling days so that caloric as well as macronutrient intake remained consistent between baseline and post. As a result, the changes in circulating hormone concentrations can be attributed to the change in energy balance and body weight as opposed to differing amounts of calories or volume of food. Lastly, the measurement of circulating hormones for a 24-hour period allowed us to account for changes in response to all three meals as well as any non-food related events that may have occurred in the profiles of these hormones. For example, the nocturnal event of ghrelin may respond to changes in sleep patterns [167, 168] and plays a role in the regulation of other hormones like cortisol and growth hormone [169]. Additionally, ghrelin and PYY are secreted peripherally from different areas of the GI tract. Ghrelin is secreted from the stomach [176] whereas PYY is mainly secreted from L cells in the ileum [2]. As exhibited in animal models, ghrelin [172] and PYY [109] are able to cross the blood brain barrier where they are believed to be involved in the hypothalamic regulation of acute and chronic energy balance. Ghrelin and PYY have been shown to activate receptors on vagal afferent neurons to modulate the regulation of energy balance in the hypothalamus from the periphery [80, 153, 177]. However, ghrelin and PYY receptors are expressed on a number of tissues in the periphery including the adrenal gland [71], adipose tissue [144] and several others. Therefore, focusing on hypothalamic regulation of ghrelin and PYY may limit the study of what these hormones may modulate, including each other, within the periphery. Measurement of peripheral signals may be a superior indication of total body regulation of the endocrine control of energy balance.
One limitation of this study is that we measured total PYY and ghrelin and not the more biologically active forms, PYY$_{3-36}$ [178] and acylated ghrelin [179]. However, it may be advantageous to capture both forms as studies have demonstrated that PYY$_{1-36}$ as well as PYY$_{3-36}$ inhibit energy intake and suppress subjective ratings of hunger [15, 129] whereas acylated ghrelin and des-acyl ghrelin stimulate energy intake [170, 180].

In conclusion, 24-hour circulating concentrations of PYY may not respond to small, but significant decreases in body weight that elicited significant increases in circulating ghrelin. Increases in circulating concentrations of ghrelin in response to diet- and exercise-induced weight loss and a weakened association between PYY and ghrelin over 24-hours, particularly at lunch, may be creating a hormonal milieu that promotes weight regain. However, there may be other endocrine factors that may be contributing to the loss of this association all of which may culminate to a signal to regain weight that has been previously lost.
Chapter 6

Hill BR, Rolls BJ, Roe LS, De Souza MJ, and Williams NI. Ghrelin and Peptide YY increase with weight loss during a 12-month intervention to reduce dietary energy density in obese women.

Abstract

Reducing dietary energy density (ED) promotes weight loss; however, underlying mechanisms are not well understood. The purpose of this study was to determine if low-ED diets facilitate weight loss through actions on ghrelin (GHR) and peptide YY (PYY), independent of influences of psychosocial measures. Seventy-one obese women (BMI 30-40kg/m\(^2\)) ages 22-60y received counseling to reduce ED. Fasting blood samples were analyzed for total GHR and total PYY by radioimmunoassay at mo 0, 3, 6, and 12. Restraint, disinhibition, and hunger were assessed by the Eating Inventory. Body weight (-7.8 ± 0.5kg), BMI (-2.9 ± 0.2kg/m\(^2\)), body fat (-3.0 ± 0.3%), and ED (-0.47 ± 0.05kcal/g or -1.97 ± 0.21kJ/g) decreased from mo 0 to 6 (p<0.05) after which no change occurred from mo 6 to 12. GHR increased in a curvilinear fashion (mo 0: 973 ± 39, mo 3: 1024 ± 37, mo 6: 1109 ± 44, and mo 12: 1063 ± 45pg/ml, p<0.001) and PYY increased linearly (mo 0: 74.2 ± 3.1, mo 3: 76.4 ± 3.2, mo 6: 77.2 ± 3.0, mo 12: 82.8 ± 3.2pg/ml, p<0.001). ED, body weight, and hunger predicted GHR, with ED being the strongest predictor (GHR = 2674.8 + 291.6 x ED - 19.2 x BW – 15 x H; p<0.05). There was a trend toward a significant association between ED and PYY (PYY = 115.0 - 43.1 x ED; p=0.05). Reductions in ED may promote weight loss and weight loss maintenance by opposing increases in ghrelin and promoting increases in PYY.
Introduction

Dietary energy density (ED; kcal/g or kJ/g) can be reduced by decreasing the proportion of fat or by increasing the water content of foods [27, 28]. Several studies [181-183] have demonstrated that humans tend to consume a consistent weight or volume of food from day to day. Thus, consuming a low-ED diet can reduce energy intake while maintaining the volume of food eaten. Decreasing dietary ED has been shown to be useful in long-term weight loss [29-33]; however, the underlying physiological mechanisms remain to be elucidated.

Specifically, no studies have examined the association between dietary ED and secretory profiles of key gut hormones involved in food intake regulation such as ghrelin and peptide YY (PYY). Ghrelin is an orexigenic hormone secreted into the blood from the X/A-like cells in the stomach and duodenum [172, 176]. Circulating ghrelin increases with weight loss in normal weight women [12, 34], whereas concentrations are suppressed and normalized (increased) with weight loss in obese individuals [35].

PYY is a satiety hormone secreted from L-cells in the distal gut where it slows digestion to increase absorption of nutrients [118]. PYY concentrations are suppressed and increased energy intake is needed to stimulate equivalent PYY secretion in obese individuals in comparison to normal weight counterparts [7]. With weight loss, circulating PYY does not necessarily return to concentrations observed in normal weight counterparts. Some studies have demonstrated increases [9, 36] whereas others have observed decreases [37-39] or no change [40] in PYY in response to weight loss. The current study adds to the literature on the role of PYY in weight loss.

Ghrelin and PYY may be related to psychosocial measures of eating behaviors such as dietary restraint (tendency to consciously restrict food intake to control body
weight) [41, 42], disinhibition (loss of control over eating in response to emotional or social cues) [43, 44], and tendency toward hunger [45]. For example, higher circulating ghrelin has been associated with high dietary restraint in obese individuals [41] and higher scores for hunger independent of BMI in a large population of normal weight individuals [45]. These studies have begun to relate behavioral to physiological measures; however, interactions between these factors have not been determined. It is attractive to posit that altered physiological states, such as suppressed concentrations of PYY, may be modulating behavioral changes, such as increases in dietary restraint, to offset biological abnormalities that may influence meal-related hunger and satiety.

The current study is an extension of a previous year-long clinical trial in which obese women received instruction in reducing dietary ED to promote weight loss [30]. Dietary ED was a significant predictor of body weight during the weight loss phase of the trial. Blood samples collected during the trial provided an opportunity to investigate how physiological mechanisms such as changes in gut hormones in response to modifications in dietary ED may promote weight loss and weight maintenance. Additionally, we sought to determine if the impact of ED on gut hormone concentrations remains significant when accounting for changes in psychosocial variables. Thus, the purpose of this study was to determine whether low-ED diets might facilitate weight loss through actions on circulating concentrations of ghrelin and PYY, independent of the influence of psychosocial measures. We hypothesized that: 1) ghrelin would increase in accordance with weight loss, but that low-ED diets would be associated with lower circulating concentrations of ghrelin, 2) PYY would decrease in response to weight loss as a
compensatory mechanism to regain weight, and 3) associations between ghrelin and ED would be independent of the influence of psychosocial variables.

Materials and Methods

Study Protocol and Participants

This study is secondary analysis of data from a randomized clinical trial that assessed the effect of reducing dietary ED as a weight loss strategy in obese women [30] (NCT ID: 00108784). Inclusion in the study was based upon the following criteria: 1) women ages 20 – 60 years 2) body mass index (BMI) between 30 – 40 kg/m². Exclusion was based upon the following criteria: 1) blood pressure > 140/90 mmHg 2) serum triacylglycerols > 400 mg/dL 3) total cholesterol > 90th percentile for age 4) the presence of medical conditions that precluded participation or condition limiting physical activity 5) pregnancy/lactation 6) taking selective serotonin reuptake inhibitors 7) symptoms of depression or disordered eating 8) currently participating in a weight loss program. All participants signed an informed consent to participate. The protocol was approved by the Institutional Review Board of The Pennsylvania State University.

Participants in the clinical trial were randomly assigned to one of two intervention groups. One group was counseled on reducing dietary fat intake to decrease the ED of their diet, and the second group was advised to increase their intake of water-rich foods such as fruits and vegetables in addition to reducing fat intake. The trial consisted of two phases: in the weight loss phase (months 0 to 6), participants attended weekly counseling sessions with a dietitian and in the weight maintenance phase (months 6 to 12), participants attended one monthly group session and one monthly individual session with a dietitian. Although the two intervention groups were counseled on different strategies to
reduce ED, all participants significantly reduced dietary ED and lost a substantial amount of weight during the trial [30]. As well, circulating concentrations of total ghrelin and total PYY at all time points (month 0, 3, 6 and 12), analysis of which were main outcome measures of the current study, were not significantly different between the two original study groups. Thus, for the purpose of characterizing hormones in the current study, the data from participants in both intervention groups who completed the year-long trial were combined for analysis.

**Intervention**

The main objective for the intervention was to reduce dietary ED in free-living obese women to determine the effect of low-ED diets on weight loss and weight loss maintenance. Participants were advised to reduce fat intake and increase intake of water-rich foods, especially fruit and vegetables. Registered dietitians led counseling sessions where participants were taught to choose foods lower in ED and appropriate in portion size. No counseling was provided on energy restriction and participants were instructed to eat ad libitum amounts of food while following the principles of the diet.

**Phase 1**

Details regarding the original clinical trial have been previously published [30]. Briefly, to achieve a reduced-fat diet, participants were provided educational materials in regard to cooking/recipe modifications, grocery shopping and dining-out strategies. Participants were taught recommended serving sizes, appropriate portion sizes and were instructed to increase water-rich foods like fruits, vegetables, and soups. They were encouraged to eat larger, satisfying portions of low-ED foods and recommended serving
sizes of medium- and high-energy density foods. Participants were also provided
behavior therapy recommendations based on social cognitive theory [184].

Phase 2

Phase 2 of the study occurred during months 6 through 12. Throughout phase 2
participants met to review material presented during phase 1. Group lessons led by
dietitians were comprised of 6 topics: holiday eating, cooking and recipe modification,
appropriate portion sizes, label reading, dining out, and grocery shopping. During
monthly individual sessions, dietitians met with participants to review 3-day diet records
and discuss any questions or concerns.

Assessment of Study Outcomes

Diet Composition

Participants attended one training session at the beginning of the study for
instruction on recording foods and beverages. Participants completed detailed three-day
diet records for two weekdays and one weekend day at each of the four time points.
Dietitians reviewed diet records with the participants to promote completeness and
accuracy. The diet records were analyzed by the Diet Assessment Center at The
Pennsylvania State University using the Nutrition Data System for Research (Nutrition
Coordinating Center, University of Minnesota, Minneapolis, MN). The analysis of energy
intake data and detailed calculation of dietary ED and fruit and vegetable intake have
been previously described [30].

Changes in Body Weight and Body Composition

Participants were weighed to the nearest 0.1 kg without shoes and while wearing
light clothing on a calibrated scale. Although body weight was measured frequently
during the trial, for comparison with the satiety hormone data, the present analyses include only the weights measured at months 0, 3, 6, and 12. Height to the nearest 0.5 cm was measured at month 0 and confirmed at month 6. Body composition measures were obtained at months 0, 3, 6, and 12. Body fat percentage (within 0.1 %) was measured by using bioelectrical impedance (Biodynamics model 310; Biodynamics Corporation, Seattle, WA) after a 12-hour fast and 48-hour abstinence from alcohol.

**Blood Sampling Procedures**

Fasting blood samples were obtained at months 0, 3, 6, and 12 of the study. Approximately 28 milliliters (mL) were collected by venipuncture at month 0 and 13.5 mL at all other time points. Serum samples were aliquoted to 2 mL microtubes and stored at – 80 ºC until analysis.

**Ghrelin and PYY Analysis**

Serum sample analysis consisted of assaying fasting blood samples for gut hormones at each time point: 0, 3, 6, and 12 months. Ghrelin was assayed using a radioimmunoassay (RIA) for total ghrelin (pg/ml) (GHRT-89HK; Millipore, Billerica, MA). The sensitivity of the assay is 93 pg/mL and the intra- and inter-assay coefficients of variation were 10.0 % and 14.7 %, respectively. PYY was assayed using an RIA for total PYY (pg/ml) (PYYT-66HK; Millipore, Billerica, MA). The sensitivity of the assay is 10 pg/mL and the intra- and inter-assay coefficients of variation were 9.4 % and 8.5 %, respectively. Samples were assayed in duplicate and all samples from a given participant were assayed within the same kit.

**Psychosocial Factors**
Participants completed the Eating Inventory [155] at months 0, 6, and 12 to measure dietary restraint (the tendency to consciously restrict food intake to control body weight), disinhibition (the loss of control over eating in response to emotional or social cues), and tendency toward hunger (the degree of subjective feelings of hunger).

**Visual Analogue Scales**

Hunger and fullness were rated immediately before and after each meal (breakfast, lunch, and dinner) on the first day that participants completed diet records using a 100-mm visual analogue scales (VAS). For example, hunger was assessed with the question “How hungry did you feel today?” The scale was anchored on the left by “not at all hungry” and on the right by “extremely hungry.” Participants also rated daily hunger and fullness 1.5 hours after their evening meal on each of the three days that the participants completed their diet records to obtain a 3-day mean.

**Statistical Analysis**

Prior to analysis, the data for ghrelin and PYY were tested for outlying values using box-plot analyses. Extreme outliers represented a value more than 3 times the inter-quartile range (Q3-Q1) from the upper (Q3) or lower (Q1) quartile. Only observations that were determined to be extreme outliers were excluded from analyses. For ghrelin, 3 observations (1 observation at month 3 and 2 observations at month 12) were excluded from 2 participants, and for PYY, one observation was excluded from one participant at baseline.

Data were screened for age-related differences in ghrelin and PYY as participants included in the current analysis were of a wide age range (22 – 60 years). Pearson correlation analyses between age and ghrelin and PYY concentrations at month 0 were
performed. Additionally, participants were divided into two groups to determine if hormone concentrations vary with age: one group including participants above the median age of menopause (51 years) [185, 186] and one group including participants below the median age of menopause. In support, one study demonstrated no difference in PYY concentrations during fasting or subsequent to intraduodenal infusions of a glucose or lipid solution between individuals in two groups, one in which ages ranged from 65 – 80 years and a second group in which individuals ranged from 20 – 34 years [187].

A linear mixed-effect model with repeated measures using a random coefficients approach was employed to determine if changes in individual outcome variables were significant over time. Time (study month) was included as a continuous covariate in the model. The month 0 value was considered to be the first repeated measurement in the model. Quadratic and cubic factors of time were tested and included if they were significant [175]. The random coefficients model accounts for the correlation of the repeated measures within participants by allowing the intercept and longitudinal response to vary randomly for each individual; thus a response curve is generated for each subject [175]. The outcomes of body weight, body composition, ED and its components (energy in kcal and weight of food in g) as well as circulating concentrations of total ghrelin and total PYY were characterized individually across all four time points.

For the main outcome variables of ghrelin and PYY we also evaluated several variables to determine significant predictors of these hormones over time by including in the statistical model measures of dietary intake, body weight and psychosocial variables [175]. Variables that were found to have significant bivariate correlations with main outcome variables (ghrelin and PYY) across the intervention or those that have been
previously reported to be associated were tested as covariates in a stepwise manner in the random coefficients model.

Post-hoc analysis to determine which variables accounted for most of the variance in ghrelin included calculating predicted outcome variable values of the full model followed by calculations of predicted values of reduced models (full model when removing one significant predictor variable from the model at a time). The sum of squares for the full model and each of the reduced models was calculated. The change in the sum of squares from the full to each of the reduced models was compared to determine the variance accounted for by the removed variable [188]. A p-value < 0.05 was considered statistically significant. Data are reported as mean ± SEM and all analyses were performed using SPSS software (Version 18.0; Chicago, IL).

Results

Participants

Participant characteristics at month 0 of the intervention are presented in Table 6.1. Participants were obese women ranging in age from 22 to 60 years. Seventy-one participants completed the original trial and were included in the current analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.7 ± 1.0</td>
<td>22.2 – 60.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.7 ± 0.6</td>
<td>153.5 – 183.0</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>90.4 ± 1.1</td>
<td>73.0 – 122.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.3 ± 0.3</td>
<td>29.6 – 40.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>39.0 ± 0.3</td>
<td>32.3 – 44.7</td>
</tr>
<tr>
<td>Body Fat (kg)</td>
<td>35.4 ± 0.6</td>
<td>24.8 – 53.4</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>55.0 ± 0.6</td>
<td>45.2 – 68.8</td>
</tr>
<tr>
<td>Restraint Score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2 ± 0.4</td>
<td>2 – 19</td>
</tr>
<tr>
<td>Hunger Score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 ± 0.4</td>
<td>0 – 14</td>
</tr>
<tr>
<td>Disinhibition Score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9 ± 0.4</td>
<td>2 – 16</td>
</tr>
</tbody>
</table>

<sup>a</sup>Eating Inventory Scores (Stunkard 1985). n = 71
Trial Outcomes

Energy Density and Energy Intake

Dietary ED decreased significantly across the intervention, as did each of its components: energy intake and food weight (Table 6.2). The random coefficients analysis found that ED followed a curvilinear relationship over time (ED = 1.76 – 0.15 x month + 0.01 x month²; p < 0.001). ED, energy intake and food weight decreased significantly from month 0 to month 3 (p < 0.001 for all), after which no significant change occurred from month 3 to month 12 (p > 0.05).
Table 6.2. Energy intake, body composition and Eating Inventory scores across the intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Month 0</th>
<th>Month 3</th>
<th>Month 6</th>
<th>Month 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>1886 ± 51</td>
<td>1366 ± 43a</td>
<td>1351 ± 41a</td>
<td>1371 ± 43a</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>7891 ± 213</td>
<td>5715 ± 180a</td>
<td>5653 ± 172a</td>
<td>5736 ± 180a</td>
</tr>
<tr>
<td>Food Weight (g)</td>
<td>3055 ± 82</td>
<td>2721 ± 94a</td>
<td>2711 ± 91a</td>
<td>2774 ± 110a</td>
</tr>
<tr>
<td>Energy Density (kcal/g)</td>
<td>1.80 ± 0.04</td>
<td>1.31 ± 0.04a</td>
<td>1.33 ± 0.05a</td>
<td>1.41 ± 0.04a</td>
</tr>
<tr>
<td>Energy Density (kJ/g)</td>
<td>7.53 ± 0.17</td>
<td>5.48 ± 0.17a</td>
<td>5.56 ± 0.21a</td>
<td>5.90 ± 0.17a</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>90.4 ± 1.1</td>
<td>84.7 ± 1.1a</td>
<td>82.6 ± 1.1ab</td>
<td>83.3 ± 1.3abc</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.3 ± 0.3</td>
<td>31.2 ± 0.3a</td>
<td>30.4 ± 0.3ab</td>
<td>30.7 ± 0.4ab</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>39.0 ± 0.3</td>
<td>37.3 ± 0.3a</td>
<td>36.0 ± 0.4ab</td>
<td>36.4 ± 0.4ab</td>
</tr>
<tr>
<td>Body Fat (kg)</td>
<td>35.4 ± 0.6</td>
<td>31.8 ± 0.6a</td>
<td>30.0 ± 0.7ab</td>
<td>30.6 ± 0.8ab</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>55.0 ± 0.6</td>
<td>52.9 ± 0.6a</td>
<td>52.6 ± 0.6c</td>
<td>52.7 ± 0.6a</td>
</tr>
<tr>
<td>Weight Lost (kg)</td>
<td>-----------</td>
<td>5.7 ± 0.4a</td>
<td>7.8 ± 0.5ab</td>
<td>7.1 ± 0.7ab</td>
</tr>
<tr>
<td><strong>Eating Inventory</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Restraint Score</td>
<td>9.2 ± 0.4</td>
<td>-----------</td>
<td>14.9 ± 0.4a</td>
<td>14.4 ± 0.4a</td>
</tr>
<tr>
<td>Hunger Score</td>
<td>6.1 ± 0.4</td>
<td>-----------</td>
<td>4.3 ± 0.3a</td>
<td>4.4 ± 0.3a</td>
</tr>
<tr>
<td>Disinhibition Score</td>
<td>9.9 ± 0.4</td>
<td>-----------</td>
<td>7.2 ± 0.3a</td>
<td>7.4 ± 0.4a</td>
</tr>
<tr>
<td><strong>VAS Scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger 3d mean</td>
<td>51.1 ± 1.9</td>
<td>50.9 ± 2.1</td>
<td>48.0 ± 2.2</td>
<td>52.5 ± 2.3c</td>
</tr>
<tr>
<td>Hunger AUC</td>
<td>486.4 ± 19.5</td>
<td>460.0 ± 16.7</td>
<td>467.0 ± 20.8</td>
<td>496.2 ± 20.3</td>
</tr>
<tr>
<td>Fullness 3d mean</td>
<td>62.0 ± 1.9</td>
<td>64.1 ± 1.9</td>
<td>62.6 ± 2.0</td>
<td>57.8 ± 2.1</td>
</tr>
<tr>
<td>Fullness AUC</td>
<td>536.0 ± 19.8</td>
<td>533.5 ± 18.0</td>
<td>538.6 ± 20.4</td>
<td>515.1 ± 17.9</td>
</tr>
</tbody>
</table>

Data are reported as Mean ± SEM, n = 71. aSignificant difference from baseline (p<0.05), bSignificant difference from Month 3 (p<0.05), cSignificant difference from Month 6 (p<0.05). Eating Inventory Scores (Stunkard 1985)
**Body weight and Body Composition**

Analysis of body weight at the four selected time points showed significant decreases across the intervention (Table 6.2), consistent with the findings reported previously for the two subject groups. The random coefficients analysis found that body weight followed a curvilinear relationship over time (Weight = 90.4 – 2.1 x month + 0.13 x month^2; p < 0.001). Table 6.2 illustrates that significant changes also occurred in other body composition parameters. The random coefficients model demonstrated that body weight (kg), body fat (kg), percent body fat, and BMI (kg/m^2) decreased significantly from month 0 to month 6 of the intervention (p < 0.001). From months 6 to 12 of the intervention, there was a trend toward a significant increase in body weight (p = 0.050); however, body weight at month 12 remained significantly lower than at month 0 (p < 0.001).

**Ghrelin**

Figure 6.1A illustrates fasting concentrations of total ghrelin at months 0, 3, 6, and 12 of the intervention. Ghrelin exhibited a significant quadratic curvilinear relationship over time (ghrelin = 967 + 33.0 x month – 1.93 x month^2; p < 0.001). The mean concentration of ghrelin increased from 973 ± 39 pg/ml at month 0 to 1109 ± 44 pg/ml at month 6, a mean increase of 134 ± 20 pg/ml or 14%. Between months 6 and 12, there were no significant changes in ghrelin.

Significant predictors of ghrelin concentrations across the intervention are presented in Table 3. No significant correlations were detected between age and ghrelin at month 0 and no differences were detected in ghrelin or PYY concentrations between participants above or below the median age of menopause and thus, age was not included.
as a covariate in the model to predict ghrelin. ED was a significant predictor of ghrelin when the statistical model included only ED as a sole independent predictor as well as when ED was included in the full statistical model that included variables known to be associated with ghrelin (full model included energy density, body weight, hunger, dietary restraint, and PYY). Post-hoc analysis comparing the change in the sum of squares between the full and reduced models revealed that ED was the strongest predictor of ghrelin, such that participants with diets lower in ED had lower circulating concentrations of ghrelin. Body weight and the Eating Inventory score for hunger were both significant negative predictors of ghrelin. The dietary restraint score was not found to be a significant predictor of ghrelin.
Figure 6.1. Results of linear mixed modeling demonstrating change in fasting concentrations and body weight from month 0 to months 3, 6 and 12 of the intervention. A. Fasting ghrelin (pg/ml) vs. body weight (kg) B. Fasting PYY (pg/ml) vs. body weight (kg). Results are reported as mean ± SEM.
Table 6.3. Significant predictors of fasting ghrelin and PYY utilizing a linear mixed effects model analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Ghrelin (pg/ml)</th>
<th>PYY (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>(95% C.I.)</td>
</tr>
<tr>
<td>Intercept</td>
<td>2674.8*</td>
<td>(2157.0, 3192.6)</td>
</tr>
<tr>
<td>ED (kcal/g)</td>
<td>69.7*</td>
<td>(6.8, 132.5)</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>-19.2*</td>
<td>(-27.9, -10.5)</td>
</tr>
<tr>
<td>Restraint Score</td>
<td>-2.1</td>
<td>(-9.3, 5.1)</td>
</tr>
<tr>
<td>Hunger Score</td>
<td>-15.0*</td>
<td>(-25.4, -4.6)</td>
</tr>
<tr>
<td>PYY (pg/ml)</td>
<td>0.2</td>
<td>(-1.0, 1.4)</td>
</tr>
</tbody>
</table>

*p<0.05, ED = Energy Density, Fruit and Veg = grams of fruit and vegetable intake excluding beverages and dried/fried sources.
PYY

Figure 1B illustrates fasting concentrations of total PYY at months 0, 3, 6, and 12 of the intervention. In contrast to the curvilinear changes in body weight, PYY increased linearly over the trial. The random coefficients model for PYY illustrated a significant linear relationship of PYY over time (PYY = 74.1 + 0.67 x month; p = 0.002). The mean concentration of PYY increased from 74.2 ± 3.1pg/ml at month 0 to 82.8 ± 3.2pg/ml at month 12, a mean increase of 8.6 ± 2.3pg/ml or 11.6%.

No significant correlations were detected between age and PYY at month 0 and no differences were detected in ghrelin or PYY concentrations between participants above or below the median age of menopause and thus, age was not included as a covariate in the model to predict PYY. There was a trend toward a significant association between dietary ED and PYY (p = 0.05), which was the only predictor included the model (full model included energy density, ghrelin, BMI, dietary restraint, disinhibition, hunger, and fruit and vegetable intake) that was associated with changes in PYY across the intervention (Table 3). The relation between ED and PYY was negative, such that diets lower in ED were associated with higher total circulating PYY. Reported fruit and vegetable intake exhibited a trend (p = 0.07) toward a significant negative association with PYY. Body mass index and the scores for dietary restraint and disinhibition were not found to be significant predictors of PYY.

Psychosocial Variables

Table 2 illustrates changes that occurred in Eating Inventory scores for dietary restraint, disinhibition, and hunger. Analysis found that these scores exhibited significant quadratic curvilinear relationships over time (p < 0.001). Scores for dietary restraint
increased from month 0 to month 6 (p < 0.001) after which there was a trend toward a significant decrease from month 6 to 12 (p = 0.06). In contrast, scores for disinhibition and hunger decreased from month 0 to month 6 (p < 0.001). No change was observed in scores for disinhibition and hunger from month 6 to 12 (p > 0.05) and scores at month 12 remained significantly lower than month 0 (p < 0.05). When considering the prediction of ghrelin by ED, ED remained a significant positive predictor of ghrelin when psychosocial variables were included in the statistical model. In addition to ED, which was the strongest predictor, the score for hunger was also a significant predictor of ghrelin (Table 3). No psychosocial variables were significant predictors when included in the statistical model predicting changes in PYY. Thus, ED alone had the strongest influence on PYY (Table 3).

**Visual Analogue Scales**

Post-dinner hunger ratings increased significantly from month 6 to 12 (p = 0.03); however, from month 3 to 6 hunger had decreased and the increase in hunger from month 6 to 12 was not significantly different from that observed at months 0 and 3. No other significant changes were observed in either hunger or fullness as measured by the 3 day mean of post-dinner hunger ratings or 3-meal AUC across the intervention (Table 6.2). As well, no significant associations were observed between VAS measures of appetite and ghrelin or PYY and thus, these variables were not included in the mixed-effects modeling analyses.

**Discussion**

Results of this study suggest that low-ED diets are associated with a hormonal milieu that likely facilitates long-term weight loss and weight loss maintenance.
Specifically, low-ED diets were associated with lower circulating concentrations of ghrelin as well as a trend toward higher circulating concentrations of PYY. The association between ED, ghrelin, and PYY persisted when accounting for psychosocial measures of eating behavior. To our knowledge, this is the first study to examine the effect of low-ED diets on alterations in gastrointestinal hormone profiles during weight loss and weight loss maintenance.

Ghrelin is, to date, the only known “hunger hormone” and its role in both short- and long-term energy balance has been well characterized [10, 12, 34, 164, 174, 189-191]. Weight loss has consistently been shown to result in elevations in circulating concentrations of fasting as well as pre- and postprandial ghrelin in both lean [12, 34] and obese participants [174]. As expected in the present trial, circulating concentrations of ghrelin increased in response to weight loss during the 12-month intervention. Though increases in ghrelin were observed, our results indicated an association between ED and ghrelin such that diets lower in ED were associated with lesser increases in circulating concentrations of ghrelin during periods of both weight loss and weight maintenance. This association was evident even when accounting for psychosocial variables that have been identified as having an impact on ghrelin [45]. Thus, low-ED diets may be promoting weight loss and weight maintenance by blunting increases in circulating concentrations of ghrelin in response to weight loss. However, mechanisms underlying the effect of low-ED diets on the attenuation of increases in ghrelin usually associated with weight loss require further investigation.

The role of PYY in relation to changes in body weight remains unclear, as some studies have shown PYY decreases in response to weight loss [37-39], whereas others
have shown increases [36, 152] or no change [40]. Our results indicate that PYY increased linearly during periods of both weight loss and weight maintenance in obese women who were following a low-ED diet. We also found that there was a trend toward a significant association between dietary ED and PYY over time. This finding suggests that low-ED diets may promote increases in circulating concentrations of PYY during a period of weight loss and subsequent weight maintenance and even when circulating concentrations of ghrelin are increasing. Though, speculation with regard to the physiological association between low-ED diets and PYY should be interpreted cautiously as the detected association did not achieve statistical significance.

It is interesting to postulate that the observed increase in PYY across our intervention, may be related to the properties of ED. PYY has traditionally been characterized as having a positive association with energy intake and particularly the macronutrients of fat and protein [3, 4]. Participants in the current trial decreased energy as well as dietary fat intake [30]. Consequently, dietary ED may be one important and unique factor involved in the modulation of PYY. PYY is believed to be involved in the slowing of gastric emptying and gastric acid secretion [2]. Our data exhibited a trend toward a significant association between low-ED diets and increases in PYY over an entire year and thus, one possible mechanism through which low-ED diets may promote chronic weight loss and weight maintenance is through promoting increases in PYY and consequent decreases in gastric acid secretion and emptying which may lead to meal cessation and perhaps, postprandial satiety [192]. However, these results should be interpreted carefully as the current study was not designed to measure gastric acid secretion or emptying and thus, one can only speculate with regard to the impact of
changes in PYY on meal cessation and satiety. Additionally, no significant predictors of PYY other than ED were detected in our statistical model. Consequently, it may be that other factors not measured in the current study impact changes in PYY in response to the dietary modification and weight loss observed herein.

Previously, it has been demonstrated that psychosocial variables such as dietary restraint, disinhibition, and hunger may impact circulating concentrations of ghrelin [45] and PYY [43]. We have demonstrated that scores for dietary restraint increased whereas both disinhibition and hunger decreased in response to a low-ED diet designed to induce weight loss. The changes in these psychosocial variables may have contributed to the weight loss observed; however, the current study suggests that ED may be impacting weight loss through its actions on ghrelin and PYY even when accounting for changes in psychosocial variables. ED was the strongest predictor of changes in ghrelin, more so than scores for hunger, suggesting that low-ED diets may promote lesser increases in circulating concentrations of ghrelin in response to weight loss. This association also suggests that low-ED diets may counteract changes in subjective feelings of hunger that are typically a consequence of elevated concentrations of ghrelin. These associations do not show causality and studies are needed to further address the mechanisms underlying the inter-relationships of ghrelin and PYY with psychosocial variables during weight loss and maintenance.

Strengths of this study are several-fold. The sample size was larger than most studies that analyze gastrointestinal hormones in response to changes in body weight. Additionally, many studies show short-term effects on weight loss with no follow up to determine if weight re-gain occurs. The current study shows prospective data where
participants lost weight and maintained the weight loss through an entire year. Lastly, ED was calculated from three-day diet records excluding beverages, which tend to have a lower ED than most foods and as such, the inclusion of beverages may disproportionately influence the calculation of ED [193].

One limitation of the study was the assessment of fasting concentrations of gut hormones as opposed to measurement of prandial events; however, we have shown in previously published data, that fasting concentrations of ghrelin and PYY are associated with the areas under the curve and 24-hour mean concentrations for each of these hormones [12, 20]. Consequently, the changes observed in the fasting concentrations of ghrelin and PYY in the current study may be related to changes in 24-hour profiles. Also, the measurement of total PYY and total ghrelin includes both circulating forms, PYY$_{1-36}$ and PYY$_{3-36}$ and des-acyl and acylated ghrelin, respectively. Though, PYY$_{3-36}$ and acylated ghrelin are the more biologically active forms, it may be beneficial to capture both forms of PYY and ghrelin as both forms may be physiologically relevant [15, 180, 194]. As with most long-term weight-loss trials, the assessment of dietary intakes in this study is based on self-reported data. Although participants were instructed on the accurate completion of diet records, which were reviewed by registered dietitians, it is known that such records can have several sources of error [195-197].

In conclusion, during weight loss and maintenance, low-ED diets were associated with lesser increases in circulating concentrations of the hunger hormone ghrelin and higher circulating concentrations of PYY. Reductions in ED may promote weight maintenance after a period of weight loss by suppressing increases in ghrelin in response
to an energy deficit that may encourage weight regain, as well as by promoting increases in circulating concentrations of the satiety hormone, PYY.
Chapter 7
Discussion

The primary purpose of this dissertation was to increase our understanding of the physiological role of PYY in response to both acute and chronic changes in energy balance and body weight in women. The specific goals of this dissertation were to: 1) characterize the diurnal rhythm of PYY with regard to acute dietary energy intake and explore its role in energy balance (study 1), 2) to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation during a period of weight stability (study 2) and subsequent to diet- and exercise-induced weight loss (study 3), and 3) to determine whether low-energy dense diets might facilitate weight loss through actions on circulating concentrations of ghrelin and PYY (study 4).

Notably, three of the four studies were conducted in normal weight, premenopausal women. Additionally, the participants included in these studies were weight stable for at least six months prior to their entry into the study and were likely in energy balance. When experiencing pathophysiological states or periods of energy imbalance such as obesity or weight loss, PYY and ghrelin may not be associated within the circulation in the same way as what has been observed here in normal weight individuals. As a result, these studies conducted in normal weight women may serve as a valuable reference to which results from studies involving abnormal states of energy balance can be compared.

Study 1 was designed to characterize features of the diurnal rhythm of PYY and to explore the role of PYY in energy balance in normal weight premenopausal women.
We demonstrated that PYY displays features of an energy-driven diurnal rhythm entrained by meal timing in healthy premenopausal women. Total energy and macronutrient content of the 24-hour sampling period were positively correlated to fasting PYY and all meal-related PYY parameters suggesting a meal-driven PYY response. As well, postprandial peaks were all significantly elevated above fasting which demonstrates that the diurnal rhythm of PYY is driven by meal timing as well. We also demonstrated that both total area under the curve and 24-hour mean PYY were positively correlated to resting metabolic rate (RMR), a major contributor to energy expenditure. We concluded that PYY can be characterized as having a meal-driven diurnal rhythm as illustrated by significant correlations between PYY and numerous meal-related parameters exemplifying that meal timing as well as caloric load of a meal elicit postprandial responses contributing to the 24-hour profile. PYY’s role in energy expenditure is correlated to absolute resting metabolism; however, a defining role in this area needs further attention. Our data further support the hypothesis that PYY plays a significant role in energy balance as a satiety hormone and correlate of energy expenditure. However, our data demonstrating a positive association between PYY and RMR are in contrast to a previous study that demonstrated a negative association between fasting PYY and 15-hour RMR in obese individuals [198] and thus, the role of PYY in energy expenditure remains unclear and future studies are necessary to clarify a role for PYY in energy expenditure in humans.

The purpose of Study 2 was to determine if the proposed opposing actions of ghrelin and PYY at the hypothalamus could be related to their patterns in the peripheral circulation during a period of weight stability in non-exercising, normal weight
premenopausal women. Our results support a role for PYY in the modulation of ghrelin in that circulating concentrations of total PYY were inversely associated with circulating concentrations of total ghrelin over an entire 24-hour period in normal weight, premenopausal women during a period of weight stability. We concluded that these data provide corroborative evidence of a reciprocal association between PYY and ghrelin over a 24-hour period in women and that these findings may substantiate inferences from experimental studies in humans and animals suggesting that changes in PYY may be modulating the secretion of ghrelin. However, in study 2 we administered meals to participants at predetermined times (0900, 1200, 1800 and 2100 hours) comprised of a set amount of calories (412 ± 28 kcal at breakfast, 472 ± 24 kcal at lunch, 504 ± 0 kcal at dinner and 66 ± 4 kcal at the snack) and controlled macronutrient content (55 % carbohydrates, 30 % fat, and 15 % protein). Thus, we were unable to determine if changing meal timing or meal energy content or composition would alter the profiles of the individual hormones or the association between ghrelin and PYY that we have previously characterized. Obtaining such information may aid in providing weight loss or weight gain dietary prescriptions in populations experiencing pathophysiological conditions such as obesity or anorexia, respectively. Future studies might characterize the 24-hour profiles of PYY and ghrelin over several 24-hour monitoring periods where meals of varying caloric and macronutrient content are administered at different times throughout the day. This would be advantageous to determine if these modifications alter the observed association between PYY and ghrelin and which specific meal-related or nocturnal events are impacted most by these alterations.
Study 3 was designed to determine if diet- and exercise-induced weight loss would alter the 24-hour profile of total circulating PYY and to determine if the association between 24-hour circulating concentrations of PYY and ghrelin observed during a period of weight stability (study 2) would be altered subsequent to a period of diet- and exercise-induced weight loss in normal weight, premenopausal women. We demonstrated that no change occurred in the entire 24-hour profile of total circulating PYY or any meal-related parameter of PYY in response to diet- and exercise-induced weight loss. As well, the inverse association observed during a period of weight stability (study 2) was weakened subsequent to a 3-month period of diet- and exercise-induced energy deficit producing significant weight loss where circulating concentrations of ghrelin increased despite administration of exactly the same calorie and macronutrient content of meals during the 24-hour analyses, but no change was observed in circulating concentrations of PYY. We created a variable to illustrate an index of hormonal exposure in an attempt to demonstrate the potential predominance of ghrelin over PYY in response to weight loss. To accomplish this, we characterized the ghrelin/PYY ratio over 24 hours at baseline and at post. The lunch ghrelin/PYY mean was significantly higher at post and trends toward significant increases were observed in the dinner and nocturnal ghrelin/PYY means demonstrating that the time frame during which uncoupling of the ghrelin and PYY profiles occurred was mainly throughout the lunch to dinner time period. We concluded that there may be an “uncoupling” occurring, particularly between lunch and dinner, due to changes in other physiological factors that may modulate ghrelin in response to an energy deficit and weight loss. This uncoupling may represent periods during which there is a greater exposure to ghrelin in comparison to PYY subsequent to
weight loss which may promote weight regain. Future studies might explore what factors modulate the secretory profile of ghrelin in response to weight loss to determine what stimulates elevations in circulating ghrelin in response to weight loss and the subsequent uncoupling between PYY and ghrelin observed in our data. Though significant weight loss occurred in study 3, the participants in the study were normal weight at baseline and lost $1.85 \pm 0.67$ kg body weight. Other studies have demonstrated that a greater amount of weight loss elicited significant decreases in PYY that persisted through an entire year [38]. Thus, it may be useful to capture the response in the 24-hour profile of PYY to varying amounts of weight loss to determine if a dose-response association or threshold of weight loss exists below which PYY concentrations are suppressed. This may be beneficial in designing weight loss prescriptions in obese individuals. A follow-up study would be to measure the 24-hour profile of PYY in response to differing stages of weight loss and subsequent weight gain over time to determine if changes in PYY observed in response to weight loss return to baseline values upon weight regain.

The purpose of Study 4 was to determine whether low-energy dense (ED) diets might facilitate weight loss through actions on circulating concentrations of ghrelin and PYY, independent of the influence of psychosocial measures of dietary restraint, disinhibition, and tendency toward hunger in obese women. We demonstrated that low-ED diets were associated with a hormonal milieu that facilitates long-term weight loss and weight loss maintenance. Specifically, low-ED diets were associated with lesser increases in circulating concentrations of ghrelin as well as higher circulating concentrations of PYY. The association between ED and ghrelin and PYY persisted when accounting for psychosocial measures of eating behavior. We concluded that
reductions in ED may promote weight maintenance after a period of weight loss by suppressing increases in ghrelin in response to an energy deficit that may encourage weight regain, as well as by promoting increases in circulating concentrations of the satiety hormone, PYY. However, we measured fasting concentrations of PYY and ghrelin and thus, did not capture the postprandial profiles of these hormones in response to low-ED diets. Though, fasting concentrations of ghrelin and PYY are physiologically relevant and may be used as proxy indicators of the 24-hour profiles of these hormones (study 1), it may be advantageous to determine if the associations observed in fasting PYY and ghrelin remain when observing these hormones postprandially. In future studies, it may be advantageous to measure the fasting as well as postprandial profiles of PYY and ghrelin in response to chronically administered low-ED diets and weight loss in obese women.

One limitation of this these four studies was that total PYY and total ghrelin were measured which includes both circulating forms of these hormones, i.e., PYY$_{1-36}$ and PYY$_{3-36}$ are included in total PYY measurements, and do not differentiate between the less biologically active circulating forms, PYY$_{1-36}$ and des-acyl ghrelin, and the more biologically active forms, PYY$_{3-36}$ [178] and acylated ghrelin [179]. However, it may be advantageous to capture both forms as studies have demonstrated that PYY$_{1-36}$ as well as PYY$_{3-36}$ inhibit energy intake and suppress subjective ratings of hunger [15, 129] whereas acylated ghrelin and des-acyl ghrelin stimulate energy intake [170, 180]. One specific limitation of the third study was that we were unable to distinguish between the effects of caloric restriction alone and the effects of exercise alone on the association between 24-hour PYY and ghrelin as the intervention was comprised of both a dietary and physical
activity component. Future studies may benefit from focusing on the individual impacts of both of these components on the association between PYY and ghrelin as food intake greatly influences the circulating profiles of these hormones and recent evidence suggests that exercise alone may also elicit acute changes in these hormones [199]. As well, one limitation of the fourth study was the assessment of fasting concentrations of gut hormones as opposed to measurement of prandial events; however, we have shown in previously published data, that fasting concentrations of ghrelin and PYY are associated with the areas under the curve and 24-hour mean concentrations for each of these hormones [12, 20]. Consequently, the changes observed in the fasting concentrations of ghrelin and PYY in the fourth study may be related to changes in 24-hour profiles. In addition, with most long-term weight-loss trials, the assessment of dietary intakes in the fourth study was based on self-reported data. Although participants were instructed on the accurate completion of diet records, which were reviewed by registered dietitians, it is known that such records can have several sources of error [195-197]. Lastly, all four studies were conducted in women and thus, inferences from these studies cannot be generalized to the male population. It has been demonstrated that men and women differ with regard to energy balance regulation [199] and thus, studies such as those presented herein in men may establish if the association between PYY and ghrelin over 24-hours differs from women in response to acute and chronic changes in energy balance.

Conclusions

These studies demonstrate that the 24-hour profile of PYY is driven by meal energy content as well as meal timing. Additionally, the 24-hour profile of PYY was inversely associated with the 24-hour profile of ghrelin during a period of weight stability.
suggesting that PYY may be involved in the feedback inhibition of ghrelin secretion. The association observed between 24-hour PYY and ghrelin was weakened subsequent to diet- and exercise-induced weight loss where increases in ghrelin, but not PYY were observed. There may be specific time periods, particularly between lunch and dinner, during the 24-hour period where an uncoupling occurs between the profiles of PYY and ghrelin suggesting that exposure to ghrelin is predominant. Other factors may be involved in modulating ghrelin secretion in response to weight loss that led to the uncoupling observed between PYY and ghrelin and ultimately creating a hormonal milieu that may promote weight regain. However, low energy dense diets may provide a protective effect against weight regain by acting on gastrointestinal hormones in a preferential manner, i.e., promoting lesser increases in ghrelin in response to weight loss as well as increases in PYY.
References


Appendix

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