Published in final edited form as:
Peptides. 2013 November ; 49: 81–90. doi:10.1016/j.peptides.2013.08.018.

The impact of weight loss on the 24-hour profile of circulating peptide YY and its association with 24-hour ghrelin in normal weight premenopausal women

Brenna R. Hill[a], **Mary Jane De Souza**[a], **David A. Wagstaff**[b], and **Nancy I. Williams**[a]

[a]Women's Health and Exercise Laboratory and the Department of Kinesiology, Pennsylvania State University, University Park, PA 16802

[b]Health and Human Development Consulting Group, College of Health and Human Development, Pennsylvania State University, University Park, PA 16802

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. We sought 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. 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. 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. 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.

Keywords

peptide YY; ghrelin; weight loss; premenopausal women

Corresponding author: Nancy I. Williams, ScD, 276 Recreation Hall, Penn State University, University Park, PA 16802, Phone: 814-865-9014, Fax: 814-865-1275, niw1@psu.edu.

Disclosure: The authors have nothing to disclose. All authors have approved the final submitted manuscript.

1. 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 [29] and/or signal to that same hypothalamic region through receptors on vagal afferent neurons [14]. Several hormones are involved in the intricate regulation of hunger and satiety; 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 [31] as well as within the peripheral circulation [3, 19].

Ghrelin was originally discovered as a growth hormone secretagogue [22]; however, its ability to stimulate energy intake was later discovered [42] 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 [11, 26]. In response to weight loss, ghrelin increases in both normal weight [24] and obese individuals [12] 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 [3-5, 23, 34]. Peripheral injection of PYY into male Wistar rats has led to inhibition of food intake and reduced weight gain [4]. 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 [4]. 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 [21], decreases [16, 38] and even no change [33] 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 [32]. Evidence to support this was provided by Riedeger et al. [31] 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 [15] and adipose tissue [8]. 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 [30], whereas ghrelin stimulates those same actions [27]. Chelikani et al. [10] 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 [3, 10]. 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 [19]. 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.

2. Participants and Methods

2.1. Experimental Design and Participants

This study is a secondary analysis of data from participants that completed 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 yrs, to emulate the exercise and restrictive eating patterns in which many young women engage. The original study design was as follows: During a four-week baseline period, participants were randomly assigned to one of four experimental groups: 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 (n=13) included in the present study represent a subset of the participants in the larger study who had completed 24-hour blood sampling and in whom those blood samples were analyzed for both ghrelin and PYY. All participants included in the present study had been randomly assigned to one of the five exercising groups in the larger study who were prescribed changes in food calories and exercised. On average, the thirteen participants in the current study achieved a negative energy balance of − 29.6 ± 9.6% less than their baseline energy needs. Of these thirteen participants, six were originally assigned to the exercising control group of the larger study. This exercising control group was prescribed a 30% increase in calories for dietary intake, and a 30% increase in exercise energy expenditure calories. Despite this prescription these six participants lost weight. Three of the thirteen participants in the current study were originally randomized to an energy deficit group that was prescribed a 15% reduction in dietary energy intake and a 15% increase in exercise energy expenditure, hence an overall daily energy deficit of − 30% when compared to baseline. Four of the thirteen participants in the current study were originally randomized to an energy deficit group that was prescribed a 30% reduction in dietary intake and a 30% increase in exercise energy expenditure, hence an overall daily energy deficit of − 60% when compared to baseline. When all thirteen subjects in the current study were compared based on their original groupings, weight loss between the original groups was not significantly different ($p = 0.53$) and no differences were observed in the hormone profile characteristics for PYY ($p > 0.05$), ghrelin ($p > 0.05$) or fasting leptin ($p = 0.71$). Because we previously reported that no changes occurred in the concentrations of 24-hour circulating total ghrelin after a 3 month diet and exercise intervention [24], a weight stable control group was not included in the present study.

2.2. 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/m^2. 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.

2.3. Body Composition and Aerobic Fitness

Body weight was measured wearing shorts and a tee-shirt (without shoes) and recorded to he nearest 0.1kg. 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 [7]. Maximal aerobic capacity (ml/kg/min) was determined using indirect calorimetry at baseline and post according to previously published methods [25].

2.4. Energy Balance Parameters

2.4.1. 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 [41], and RQ.

2.4.2. 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.

2.4.3. 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).

2.4.4. 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 [25]. 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.

2.5. 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 2500 rpm. Serum aliquots were transferred to storage tubes and stored in a − 80° 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.

2.6. 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 a radioimmunoassay kit (Millipore, Billerica, MA). 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.

2.7. 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.

2.8. Fasting Blood Draw

Fasting morning blood samples were collected during the early follicular phase (menstrual cycle days 1–7) of the baseline and at post between 0700 and 1000 hours at the GCRC after participants lay supine for at least 15 minutes. Participants abstained from exercise for at least 12 hours before blood sampling. Each sample was allowed to clot at room temperature for 1 hour and subsequently spun in a centrifuge for 15 minutes at 2500 rpm. Serum aliquots were transferred to storage tubes and stored in a − 80° Celsius freezer until analysis.

2.9. Leptin

For descriptive purposes, leptin was measured in duplicate in fasting serum samples from each participant at baseline and at post using a radioimmunoassay kit for total leptin (Linco Research, St. Charles, MI). The interassay and intraassay coefficients of variation for the high controls were 3.6% and 3.4 %, respectively; the interassay and intraassay coefficients of variation for the low control were 6.2% and 8.3%, respectively. The sensitivity of this assay was 0.5 ng/ml. All samples from a given subject were analyzed in duplicate, and in the same assay.

2.10. Data Analysis

2.10.1. 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).

2.11. 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 [6]. 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).

3. Results

3.1. Participants

Participants' demographic characteristics at baseline are presented in Table 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 VO_{2max} at baseline was 37.8 ± 1.4 ml/kg/min. This value is between the 50^{th} and 55^{th} percentile for women ages 20 – 29 (ACSM Guidelines 8^{th} Ed.) and likely consistent with a moderately active lifestyle. Ten (77%) participants were Caucasian, two (15%) were African-American, and one was Hispanic (8%).

3.2. Effect of the Intervention on Body Composition, Leptin and Aerobic Fitness

Table 1 also displays the change from baseline to post in body composition, leptin and aerobic fitness. There was a statistically significant decrease in body weight (− 1.85 ± 0.67 kg), BMI (− 0.69 ± 0.24 kg/m^2) and body fat (− 2.53 ± 0.83%) from baseline to post. However, the change in fat free mass was not statistically significant (0.02 ± 0.46 kg). As well, there was a statistically significant decrease in leptin from baseline to post (− 2.5 ± 1.8 ng/ml) and there was a statistically significant increase in the participants' aerobic fitness as measure by VO_{2max} (6.98 ± 1.63 ml/kg/min) from baseline to post.

3.3. Ghrelin

Figure 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 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.

3.4. PYY

Figure 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 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.

3.5. The Association between Ghrelin and PYY

Figure 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 [19]. 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 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 3).

4. 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 descriptive parameter of the 24-hour profile of circulating PYY. This is also the first account of changes in the association between 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, was weakened 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. 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.

Though studies have reported that PYY may inversely correlates with body weight, i.e. PYY concentrations are suppressed in obese individuals [23] and elevated in anorexia nervosa [28], findings with regard to how PYY in the circulation changes in response to body weight loss are not consistent, highlighting the complexity of factors modulating PYY secretion. Several studies have reported varying results of fasting or changes in single meal-related responses in postprandial PYY subsequent to weight loss [16, 21, 33, 38]. 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 [24]. 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. [33] 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. Alternatively, PYY may increase with favorable changes in body composition with no concomitant change in body weight. Jones et al. [21] demonstrated that circulating PYY increased in response to a 12 week exercise intervention that lead to significant fat, but not body weight, loss. However, this study was performed in children and thus results may not translate to 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. Moreover, studies are necessary to elucidate the mechanisms underlying the impact of changes in energy balance on PYY.

There are few studies similar to the present study that have examined the association between ghrelin and PYY in the peripheral circulation [3, 10]. Batterham et al. [3] 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. 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 [19]. 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. 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.

Weakening 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. Increases observed in ghrelin across the day may 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 [37]. 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 a 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 [35, 36] and plays a role in the regulation of other

hormones like cortisol and growth hormone [40]. Additionally, ghrelin is secreted from the stomach [13] whereas PYY is mainly secreted from L cells in the ileum [1]. As exhibited in animal models, ghrelin [22] and PYY [29] are able to cross the blood brain barrier and have been shown to activate receptors on vagal afferent neurons to modulate the regulation of energy balance in the hypothalamus from the periphery [2, 14, 43]. As well, ghrelin and PYY receptors are expressed on a number of tissues in the periphery including the adrenal gland [15], adipose tissue [8] 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 the thirteen subjects included in this study were originally randomized to different experimental groups and thus, experienced varying prescriptions to change dietary intake calories and or exercise caloric expenditure. However, a statistical analysis was performed to determine if hormonal outcomes from the study differed depending on the original group to which these subjects were assigned and no differences in weight loss and hormonal responses were found. Due to the varied prescriptions for diet and exercise, further studies are required to address whether the observed changes in ghrelin and PYY, and their relation to each other, are attributable to diet alone, exercise alone, or the combination of diet and exercise. Another limitation is that we measured total PYY and ghrelin and not the more biologically active forms, $PYY_{3\text{-}36}$ [18] and acylated ghrelin [20]. However, it may be advantageous to capture both forms as studies have demonstrated that $PYY_{1\text{-}36}$ as well as $PYY_{3\text{-}36}$ inhibit energy intake and suppress subjective ratings of hunger [9, 34] whereas acylated ghrelin and des-acyl ghrelin stimulate energy intake [17, 39].

4.1. Conclusions

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. As such, it may be relevant to further explore the increased ratio of orexigenic to anorexigenic peptides that may be promoting weight gain subsequent to a period of weight loss.

Acknowledgments

We thank Heather Leidy and the staff that spent numerous hours on the original study for their contributions. We also appreciate the cooperation of the study volunteers. Brenna R. Hill is supported by the Intercollegiate Graduate Degree Program in Physiology at the Pennsylvania State University. This work was supported by NIH Grants 1R01HD39245-01A1 and M01 RR 10732.

References

[1]. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology. 1985; 89:1070–7. [PubMed: 3840109]

[2]. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology. 2001; 120:337–45. [PubMed: 11159873]

[3]. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med. 2003; 349:941–8. [PubMed: 12954742]

[4]. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature. 2002; 418:650–4. [PubMed: 12167864]

[5]. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006; 4:223–33. [PubMed: 16950139]

[6]. Brown HaP, R. Applied Mixed Models in Medicine. 2nd ed. John Wiley & Sons, Ltd.; Chichester: 2006.

[7]. Brozek J, Grande F, Anderson JT, Keys A. Densitometric Analysis of Body Composition: Revision of Some Quantitative Assumptions. Ann N Y Acad Sci. 1963; 110:113–40. [PubMed: 14062375]

[8]. Castan I, Valet P, Larrouy D, Voisin T, Remaury A, Daviaud D, et al. Distribution of PYY receptors in human fat cells: an antilipolytic system alongside the alpha 2-adrenergic system. Am J Physiol. 1993; 265:E74–80. [PubMed: 8393293]

[9]. Chelikani PK, Haver AC, Reidelberger RD. Intravenous infusion of peptide YY(3-36) potently inhibits food intake in rats. Endocrinology. 2005; 146:879–88. [PubMed: 15539554]

[10]. Chelikani PK, Haver AC, Reidelberger RD. Ghrelin attenuates the inhibitory effects of glucagon-like peptide-1 and peptide YY(3-36) on food intake and gastric emptying in rats. Diabetes. 2006; 55:3038–46. [PubMed: 17065340]

[11]. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes. 2001; 50:1714–9. [PubMed: 11473029]

[12]. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med. 2002; 346:1623–30. [PubMed: 12023994]

[13]. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology. 2000; 141:4255–61. [PubMed: 11089560]

[14]. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, et al. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology. 2002; 123:1120–8. [PubMed: 12360474]

[15]. Ekblad E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides. 2002; 23:251–61. [PubMed: 11825640]

[16]. Essah PA, Levy JR, Sistrun SN, Kelly SM, Nestler JE. Effect of weight loss by a low-fat diet and a low-carbohydrate diet on peptide YY levels. Int J Obes (Lond). 2010; 34:1239–42. [PubMed: 20351741]

[17]. Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, Gaylinn BD, et al. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. J Clin Endocrinol Metab. 2008; 93:1971–9. [PubMed: 18198223]

[18]. Grandt D, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE, et al. Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. Regul Pept. 1994; 51:151–9. [PubMed: 8059011]

[19]. Hill BR, Souza MJ, Wagstaff DA, Sato R, Williams NI. 24-Hour profiles of circulating ghrelin and peptide YY are inversely associated in normal weight premenopausal women. Peptides. 2012

[20]. Hosoda H, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun. 2000; 279:909–13. [PubMed: 11162448]

[21]. Jones TE, Basilio JL, Brophy PM, McCammon MR, Hickner RC. Long-term exercise training in overweight adolescents improves plasma peptide YY and resistin. Obesity (Silver Spring). 2009; 17:1189–95. [PubMed: 19247279]

[22]. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999; 402:656–60. [PubMed: 10604470]

[23]. le Roux CW, Batterham RL, Aylwin SJ, Patterson M, Borg CM, Wynne KJ, et al. Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology. 2006; 147:3–8. [PubMed: 16166213]

[24]. Leidy HJ, Dougherty KA, Frye BR, Duke KM, Williams NI. Twenty-four-hour ghrelin is elevated after calorie restriction and exercise training in non-obese women. Obesity (Silver Spring). 2007; 15:446–55. [PubMed: 17299118]

[25]. Leidy HJ, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL, et al. Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. J Clin Endocrinol Metab. 2004; 89:2659–64. [PubMed: 15181038]

[26]. Leidy HJ, Williams NI. Meal energy content is related to features of meal-related ghrelin profiles across a typical day of eating in non-obese premenopausal women. Horm Metab Res. 2006; 38:317–22. [PubMed: 16718628]

[27]. Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, et al. Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun. 2000; 276:905–8. [PubMed: 11027567]

[28]. Misra M, Miller KK, Tsai P, Gallagher K, Lin A, Lee N, et al. Elevated peptide YY levels in adolescent girls with anorexia nervosa. The Journal of clinical endocrinology and metabolism. 2006; 91:1027–33. [PubMed: 16278259]

[29]. Nonaka N, Shioda S, Niehoff ML, Banks WA. Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. J Pharmacol Exp Ther. 2003; 306:948–53. [PubMed: 12750431]

[30]. Pappas TN, Debas HT, Goto Y, Taylor IL. Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. Am J Physiol. 1985; 248:G118–23. [PubMed: 3838121]

[31]. Riediger T, Bothe C, Becskei C, Lutz TA. Peptide YY directly inhibits ghrelin-activated neurons of the arcuate nucleus and reverses fasting-induced c-Fos expression. Neuroendocrinology. 2004; 79:317–26. [PubMed: 15256809]

[32]. Sam AH, Troke RC, Tan TM, Bewick GA. The role of the gut/brain axis in modulating food intake. Neuropharmacology. 2012; 63:46–56. [PubMed: 22037149]

[33]. Scheid JL, De Souza MJ, Leidy HJ, Williams NI. Ghrelin but not peptide YY is related to change in body weight and energy availability. Medicine and science in sports and exercise. 2011; 43:2063–71. [PubMed: 21502892]

[34]. Sloth B, Holst JJ, Flint A, Gregersen NT, Astrup A. Effects of PYY1-36 and PYY3-36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. Am J Physiol Endocrinol Metab. 2007; 292:E1062–8. [PubMed: 17148749]

[35]. Spiegel K, Tasali E, Leproult R, Scherberg N, Van Cauter E. Twenty-four-hour profiles of acylated and total ghrelin: relationship with glucose levels and impact of time of day and sleep. J Clin Endocrinol Metab. 2011; 96:486–93. [PubMed: 21106712]

[36]. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann Intern Med. 2004; 141:846–50. [PubMed: 15583226]

[37]. Stanley S, Wynne K, Bloom S. Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. Am J Physiol Gastrointest Liver Physiol. 2004; 286:G693–7. [PubMed: 15068960]

[38]. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. 2011; 365:1597–604. [PubMed: 22029981]

[39]. Toshinai K, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, et al. Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. Endocrinology. 2006; 147:2306–14. [PubMed: 16484324]

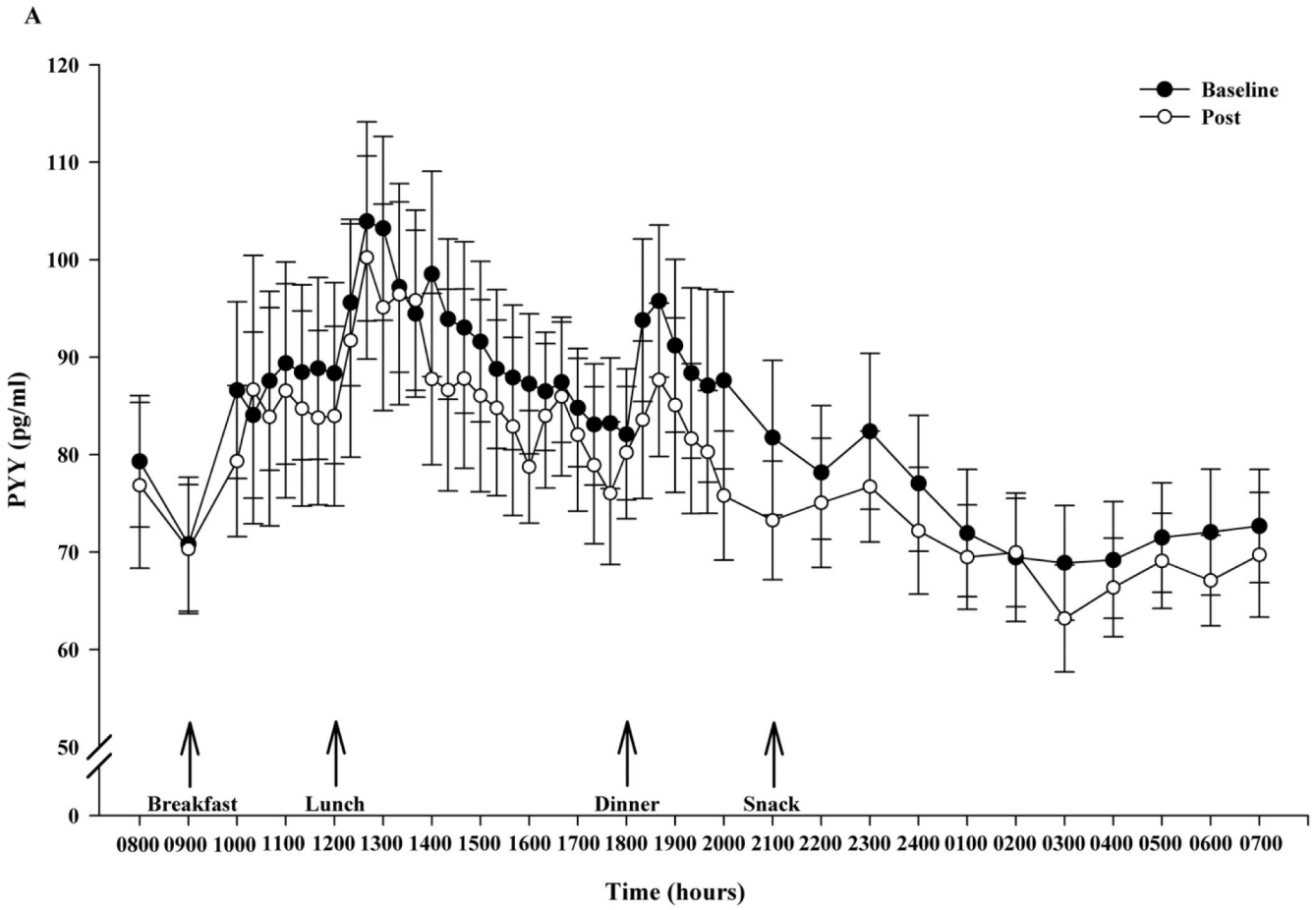
[40]. Weikel JC, Wichniak A, Ising M, Brunner H, Friess E, Held K, et al. Ghrelin promotes slow-wave sleep in humans. Am J Physiol Endocrinol Metab. 2003; 284:E407–15. [PubMed: 12388174]

[41]. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949; 109:1–9. [PubMed: 15394301]

[42]. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology. 2000; 141:4325–8. [PubMed: 11089570]

[43]. Zhang T, Uchida T, Gomez G, Lluis F, Thompson JC, Greeley GH Jr. Neural regulation of peptide YY secretion. Regul Pept. 1993; 48:321–8. [PubMed: 8278624]

- 24-hour PYY may not respond to weight loss that elicited increases in ghrelin
- Elevations in ghrelin concomitant with no change in PYY may promote weight regain
- A weakened 24-hour PYY and ghrelin association may occur particularly at lunch
- Other factors may contribute to this weakened association to encourage weight regain



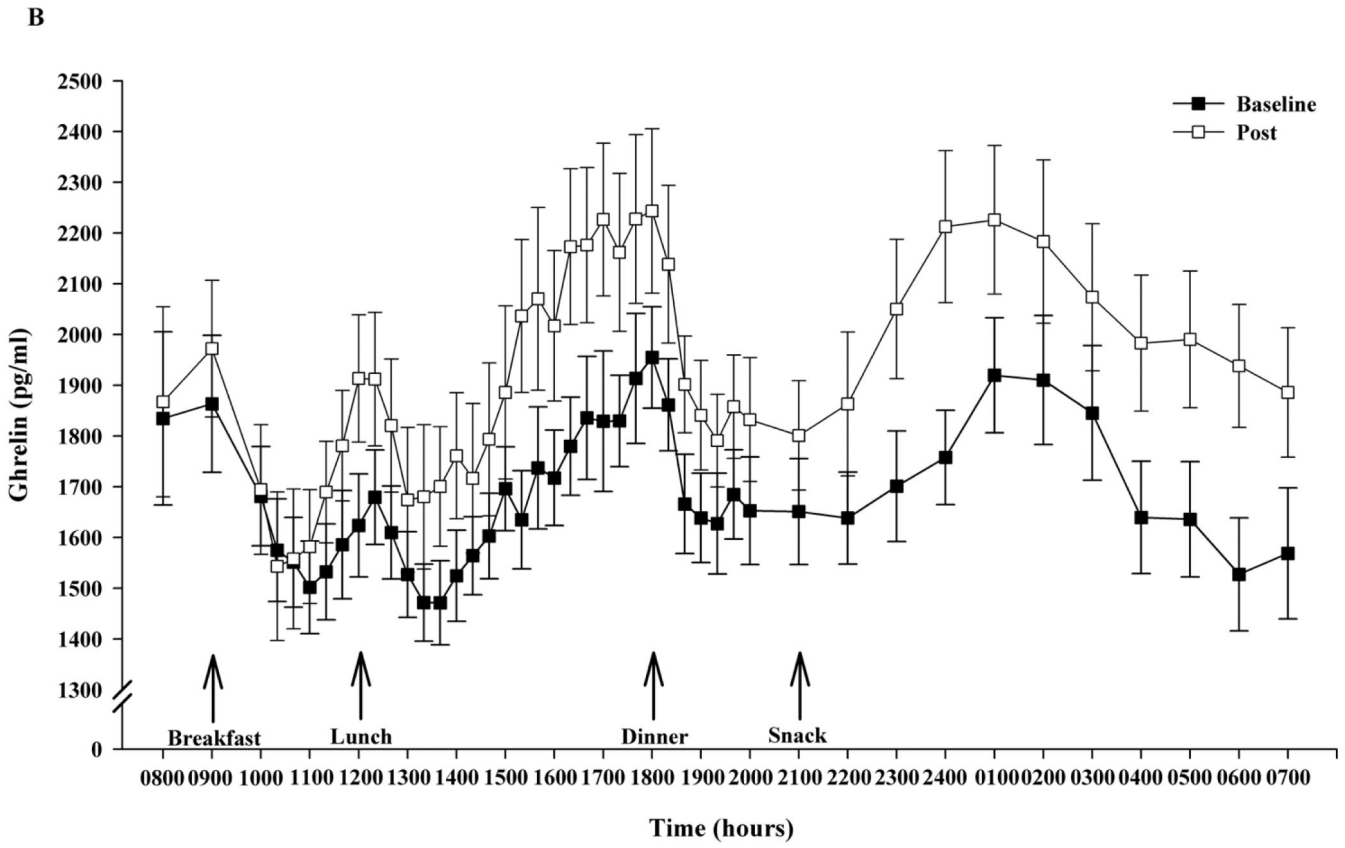
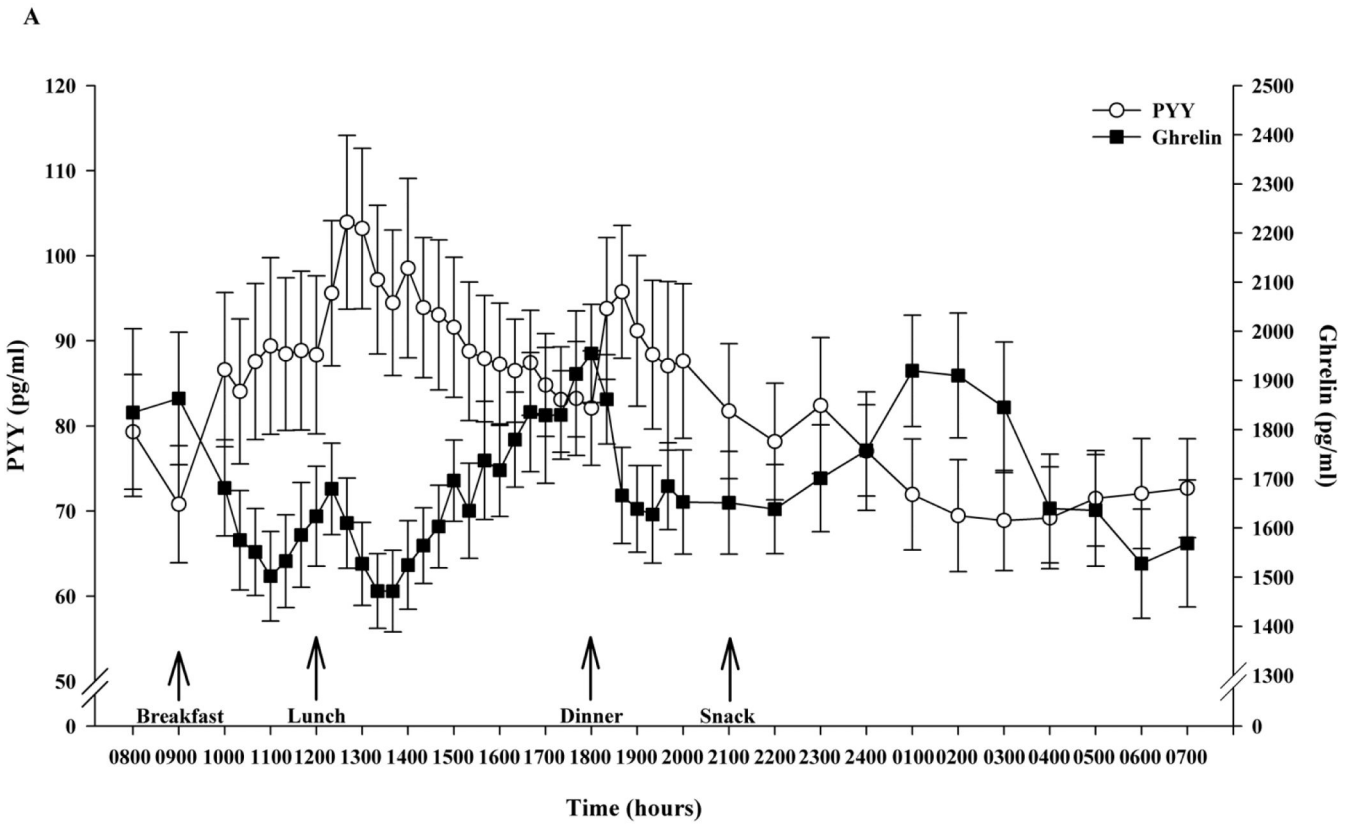
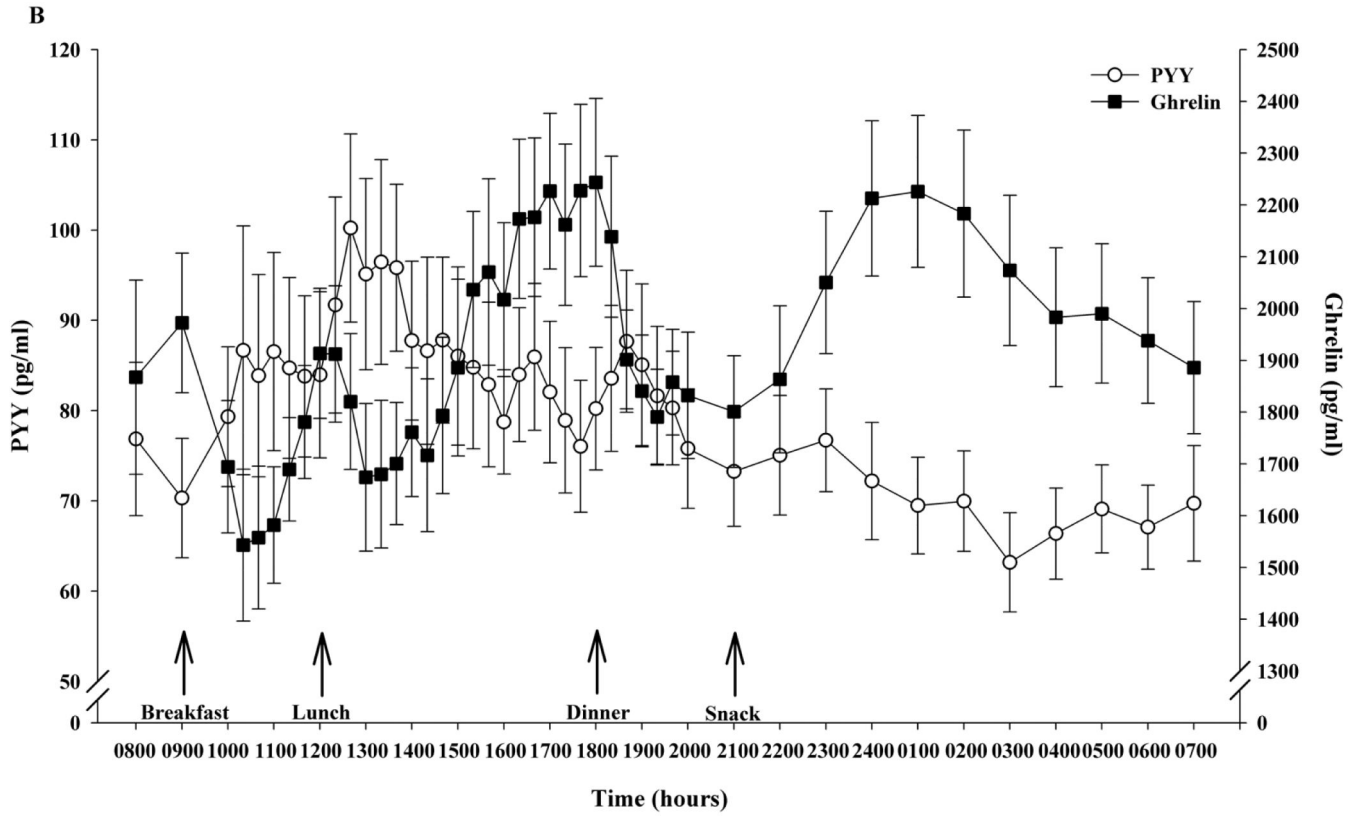


Figure 1.

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.





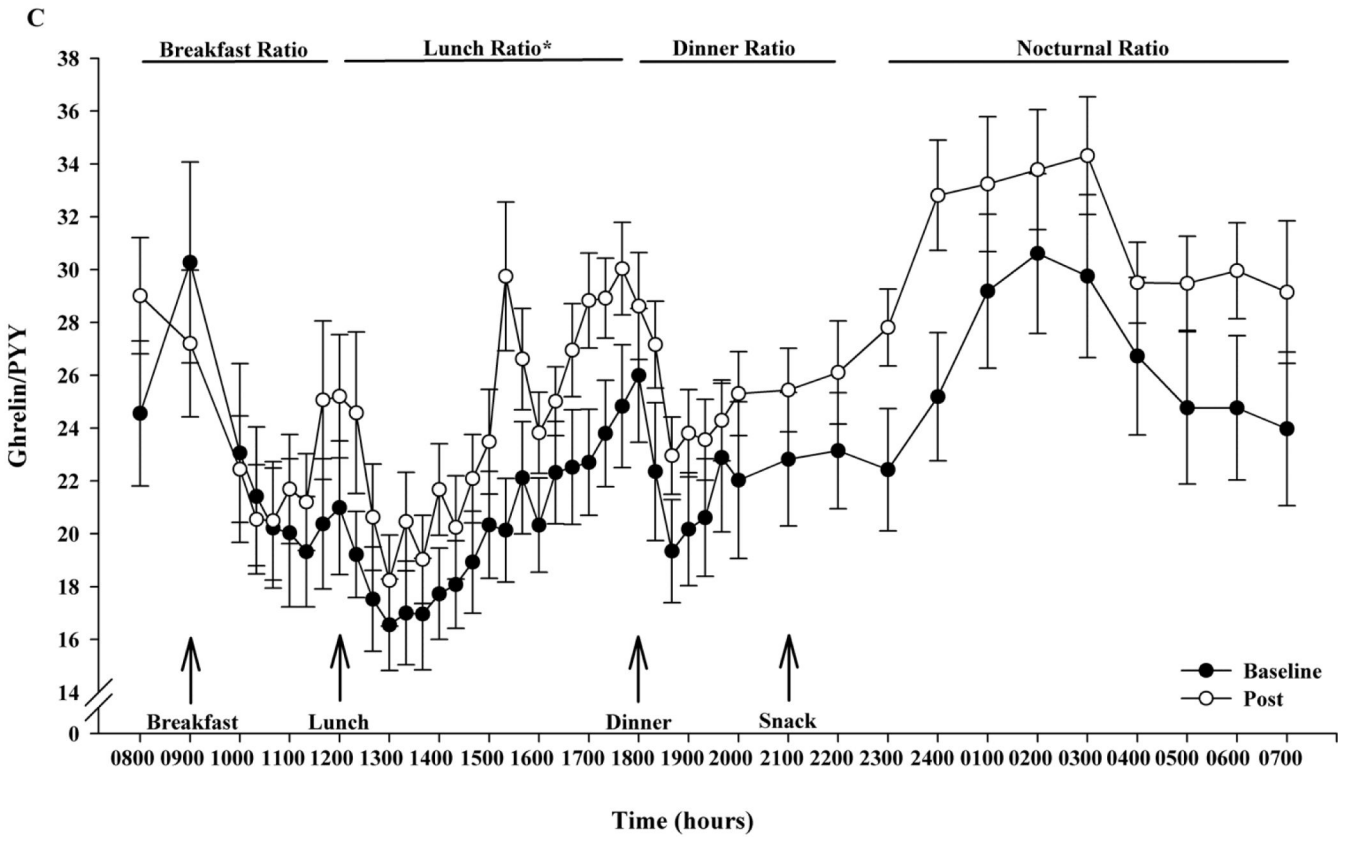


Figure 2.

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. **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 ghrelin/PYY ratio meal or nocturnal mean). Data are reported as mean + SEM; *p < 0.05

Table 1

Participant demographics at baseline and post (n=13)

Variable	Baseline (Mean ± SEM)	Range	Post (Mean ± SEM)	Range	p-value
Age (yr)	20 ± 0.5	18 – 24	20 ± 0.5	18 – 24	n/a
Height (cm)	163.7 ± 1.4	154.3 – 170.2	163.7 ± 1.4	154.3 – 170.2	n/a
Body Weight (kg)	57.0 ± 1.5	51.1 – 66.5	55.2 ± 1.4	48.0 – 65.5	0.02 *
BMI (kg/m^2)	21.3 ± 0.5	18.8 – 23.9	20.6 ± 0.5	18.0 – 23.6	0.02 *
Body Fat (%)	26.1 ± 1.5	19.0 – 36.8	23.5 ± 1.3	18.6 – 34.5	0.01 *
Fat Mass (kg)	15.0 ± 1.1	9.8 – 22.2 i	13.1 ± 1.0	9.2 – 21.2	0.01 *
Fat Free Mass (kg)	42.1 ± 1.1	37.2 – 49.6	42.1 ± 1.0	36.0 – 47.0	0.97
VO_2 max (kcal/kg/min)	3.8 ± 1.4	29.8 – 42.7	43.5 ± 1.6	36.4 – 52.9	0.002 *
Leptin (ng/ml)	8.9 ± 0.8	3.6 – 12.7	6.4 ± 0.9	2.4 – 15.7	0.01 *

* $p < 0.05$

Table 2

Baseline and post characteristics of PYY and Ghrelin

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

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.

* $p<0.05$

Table 3

Ghrelin/PYY ratio parameters at baseline and post

Ghrelin/PYY Variable	Baseline (Mean ± SEM)	Post (Mean ± SEM)	p-value
24-hour Ratio	22.2 ± 2.2	25.5 ± 1.7	0.05
Breakfast Ratio	23.3 ± 2.9	23.2 ± 2.2	0.96
Lunch Ratio	20.1 ± 1.8	24.1 ± 1.6	0.01*
Dinner Ratio	22.0 ± 2.4	25.5 ± 1.6	0.07
Nocturnal Ratio	26.4 ± 2.7	31.0 ± 2.1	0.08

* $p < 0.05$