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The Role of Exercise Dose on Ghrelin Concentration in Postmenopausal Women

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THE ROLE OF EXERCISE DOSE ON GHRELIN CONCENTRATION IN POSTMENOPAUSAL WOMEN

by

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ABSTRACT

Introduction: As obesity continues to be a major public health concern, exercise is continually at the forefront of combating this epidemic. This thesis aims to examine how two different doses of exercise may affect the “hunger hormone”, ghrelin; in postmenopausal, non-obese women. Methods: 54 non-obese postmenopausal women were randomly assigned to either a higher dose (energy expenditure = 14 kilocalories/per kilogram of body weight/per week) or lower dose (energy expenditure = 8 kcal/kg/week) aerobic exercise training group for 16 weeks. Fasting blood samples were taken at baseline and at post intervention for analysis of ghrelin via multiplex immunoassay. Results: Only the higher dose exercise group had a decrease in ghrelin concentration following 16 weeks of moderate intensity aerobic exercise. Conclusion: Higher dose aerobic exercise training significantly decreased fasting ghrelin concentration and increased VO_2 in non-obese postmenopausal women while the lower-dose chronic exercise training did not.

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LIST OF ABBREVIATIONS

ANOVA .....Analysis of Variance

BMI ..... Body Mass Index

CV.....Coefficient of Variation

ECG.....Electrocardiogram

Kg .....Kilogram

pg/ml..... pictogram per milliliter

SD..... Standard Deviation

VCO_2..... Volume of Carbon Dioxide

VO_2 ..... Volume of Oxygen

CHAPTER 1

MANUSCRIPT

Obesity is a global health problem that is associated with increased risks for several diseases such as type 2 diabetes and cardiovascular disease (1). In recent years, the prevalence of overweight and obesity in middle aged and older women has continued to increase (1). By 2025, the North American Menopause Society has determined that the population of older adults is estimated to increase considerably, and those that are overweight or obese will most likely require additional clinical care; further extending the cost of healthcare related to obesity. With this in mind, it is evident that this population requires additional attention in regards to understanding and controlling this trend (1).

Overweight and obesity most likely results from a chronic imbalance of energy intake and energy expenditure. Energy balance and body weight and composition are regulated by many factors including a complex network of hormones with energy regulating properties, such as detecting changes in the body's energy balance, relaying that information to the central processing centers in the brain, and inducing changes in energy intake/expenditure. These hormones are commonly grouped into two categories, either episodic/short term, or tonic/long term. Ghrelin is widely recognized as the main episodic regulator of energy intake (2). It plays a role in the regulation of energy balance by increasing appetite and initiating food intake, as well as reducing fat utilization

(3). Total plasma ghrelin concentrations increase pre-prandially and fall post-prandially (4); however the post-prandial decrease in ghrelin concentration is not seen in obese population (5). Ghrelin infusion studies have found a continuous infusion of exogenous ghrelin can increase appetite sensations inboth obese and lean participants (6). Ghrelin concentrations are found in circulation to be inversely proportional to body weight – higher circulating ghrelin concentrations are found in lower body weights, and lower circulating levels are found in high body weights (5, 7, 8). Following weight change, ghrelin typically responds in a compensatory fashion, with concentrations increasing with weight loss, and decreasing with weight gain (7). Studies of long term caloric restriction ranging from three to six months resulting in significant weight loss have resulted in increased ghrelin concentrations (5, 9).

Exercise as a means to induce negative energy balance and potentially weight loss has been suggested to increase fasting ghrelin concentrations and therefore, appetite sensations. However, there is evidence suggesting that the effect of exercise on ghrelin may be different from weight loss resulting from caloric restriction (4). Several previous studies have investigated the response of ghrelin concentrations to chronic exercise training; however the results of these studies are inconclusive (4, 7, 10-12). The different doses of exercise used in the studies may be a factor contributing to the inconsistent findings, but this has not been investigated in the same study. Additionally, obese individuals may have a different response to an exercise intervention from normal weight individuals (11). Research by Heden et al found normal weight individuals had a decrease

in fasting ghrelin concentration 12 hours after a one-hour moderate intensity exercise bout, but this decrease was absent in obese individuals (11). Most previous exercise training studies, however, have examined ghrelin response in obese individuals. To our knowledge, normal weight older women are a segment of the population that have yet to be studied in this capacity.

The purpose of this study was to investigate the ghrelin response to two different doses of chronic aerobic exercise training in non-obese older women without a dietary intervention. We hypothesized that in women performing the higher exercise dose would have a decrease in fasting circulating ghrelin concentration, while women performing a lower dose of exercise would not experience the decrease in fasting ghrelin concentration.

Methods

This study used data collected in the Women's Energy Expenditure in Walking Programs Study (Clinicaltrials.gov identifier: NCT01722136). The WeWalk Study was a 4-month randomized control trial to investigate the effects of moderate intensity aerobic exercise of two different doses on energy expenditure in inactive older women, with the higher-dose group meeting the current public health guidelines for the recommended amount of moderate intensity aerobic exercise per week, while the lower-dose group will fall below the recommended amount. Study procedures were reviewed and approved by the University of South Carolina institutional Review Board in Columbia, South Carolina. All participants signed a written informed consent.

Participants

The participants of the WeWalk Study were non-obese (body mass index, BMI, 18 – 30 kg/m^2) older women (60-75 years) from the Columbia metropolitan area. They were recruited through advertisements in the local newspapers, emails lists, and lunch n' learn programs. Briefly, exclusionary criteria included: medications known to affect exercise performance or metabolism, self-reported or upon medical examination, signs of metabolic disorders such as diabetes or thyroid disorders, heart disease, kidney disease, lung disease, cancer diagnoses within the last 5 years (except skin cancer), large weight fluctuations in the last 3 months (>3%), currently smoking, current participation in another intervention, physically active (greater than 20 minutes x 3 times per week of structured exercise) during the past 3 months, any contraindications to exercise, or abnormal responses during the graded exercise test (see below).

Graded exercise test

Participants completed a maximal graded treadmill test at baseline and post-intervention. The test was an incremental protocol, participants walked at a self-selected pace which remained constant throughout the duration of the test. Every 2 minutes, the incline of the treadmill was increased by 2%. Volume of O_2 (VO_2) and CO_2 (VCO_2) was measured via a metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT, USA). Heart rate via a standard 12-lead ECG (Q-Stress; Cardiac Science, Bothell, WA, USA) was monitored by a trained medical

personnel. Blood pressure readings were taken approximately 30 seconds into each stage of the test. For the test to be considered satisfactory, two of four criteria had to be met: a plateau in heart rate or VO_2; respiratory exchange ratio greater than or equal to 1.10; a maximum heart rate greater than 90% of age-predicted maximal heart rate (= 220-age); or a rating of perceived exertion greater than or equal to 17 on the Borg scale. VO_2peak was determined by the maximal 30-second averaged VO_2 value during the test.

Exercise Intervention

Participants were randomized into one of two moderate-intensity exercise groups. The lower-dose group had an energy expenditure goal of 8 kilocalories (kcal) per kilogram (kg) of body weight per week, and the higher-dose group had an energy expenditure goal of 14 kcal/kg of body weight per week. All training sessions were supervised and occurred at the Clinical Exercise Research Center at the University of South Carolina. Both groups exercised for 16 weeks at an intensity of 50-55% of the participant's heart rate reserve (HRR), which was calculated using information obtained from each woman's graded exercise test. Due to the participants being inactive prior to their participation in the study, training intensity started at 40% of HRR and was increased by 5% every two weeks until the target level of 50-55% was achieved. The exercise target was reached within 5 weeks for women in the lower dose group and 8 weeks for those in the higher dose group. Heart rate monitors (FT1; Polar; Lake Success, NY, USA) were worn during training sessions to ensure participants were exercising at the desired intensity throughout each exercise session. Heart

rate was monitored and recorded every 5 minutes throughout each training session, and blood pressure was measured before, at the midpoint of the exercise session, and after the exercise sessions. Women in the lower and higher-dose groups walked an average of 105 minutes and 160 minutes per week, respectively. Most women walked 3 times a week, although a few women in the higher-dose group walked 4 times a week.

Body Composition

The dual-energy absorptiometry (enCORE, GE Healthcare model 8743, Waukesha, WI) full body scan was utilized to measure body fat percentage, lean mass (kg), fat mass (kg) and fat free mass (kg) at baseline and post-intervention. Height was measured to the nearest 0.1cm without shoes, and weight was measured to the nearest 0.1kg while the participant was wearing lightweight scrubs. Two measurements for both height and weight were taken, and averages were used to calculate BMI (kg/m^2).

Blood Sample Collection and Analysis

Venous blood samples were collected at baseline and following the exercise intervention. Participants were instructed to fast (not including water) for at least 12 hours prior to blood draw visits. Blood samples were drawn into EDTA tubes, centrifuged at 3,000 rpm for 20 minutes at 4 degrees Celsius. Plasma was then aliquoted into Eppendorf tubes and stored at -80 degrees Celsius until analysis.

Plasma samples were thawed and re-centrifuged at 3,000 rpm at 4C° for 20 minutes to remove any potential particulates before analysis of ghrelin concentration. Plasma ghrelin was quantified by a multiplex immunoassay (Human Metabolic Hormone Magnetic Bead Panel, Millipore, Billerica, MA) per manufacturer instructions on a Magpix multiplexing system (Luminex, Austin, Texas, USA). The assay has a minimum detectable concentration of 13 pg/ml. The intra-assay coefficient of variance (CV) in our laboratory was < 10% and inter-assay CV was < 7%. All samples were run in duplicates.

Statistical Analysis

Descriptive statistics were first examined and are reported as mean ± SD. Change values in body weight, fat mass, fat-free mass, and ghrelin concentration were calculated using post intervention values subtracted from baseline values. Baseline and change values were compared between the exercise groups using one-way analysis of variance (ANOVA). Within each group, paired t-test was used to determine if values at post-intervention were different from baseline. ANOVA with repeated measures were used to determine the changes following exercise training were different between the two exercise groups. A p value of < 0.05 was used to denote statistical significance. Because of the reported association between body weight and body composition with ghrelin concentration in the literature, regression analyses were used to determine corrections between baseline and changes in body weight, fat mass, fat-free mass, and VO_2peakwith ghrelin concentration. The SAS software (version 9.4, SAS Institute, Cary, NC) were used to perform analyses.

Results

Of the 54 participants, the majority were Caucasian (89%) and 87% of the participants had at least some college education. As shown in Table 1.1, at baseline there were no significant differences between groups for age (years), height (cm), weight (kg), BMI (kg/m2), VO_2 (ml/kg/min), fat mass (kg), fat free mass (kg), percent body fat, systolic & diastolic blood pressure(mmHg), or fasting ghrelin concentration(pg/ml) between groups. In the entire sample, body weight, body composition or VO_2 peak were not correlated with ghrelin concentration ($p > 0.05$ for all).

Following the exercise training, repeated measures ANOVA revealed a significant group by time interaction for VO_2 peak [$F(1,52) = 7.09$, $p = 0.0103$] only. Table 1.2 shows change values for body weight, fat mass, lean mass, percent fat, and VO_2 by group. Within the lower dose group, there was no change in weight, fat mass, lean mass, percent body fat, or VO_2 peak from baseline to post-intervention measurements. Within the higher-dose group, following the exercise intervention, there was no change in weight, fat mass, lean mass, or percent body fat; but VO2 peak significantly increased.

As shown in table 1.3, there was no change in fasting ghrelin concentration in the lower-dose exercise group from baseline to post-intervention. However, in the higher-dose exercise group, there was a significant decrease by 27.43 ± 54.78 pg/ml in fasting ghrelin concentration from baseline to post-intervention. Figure 1.1 provides a graphical representation of the changes

in fasting ghrelin concentration from baseline to post-intervention for the higher-dose exercise group, and figure 1.2 addresses the lower-dose exercise group fasting ghrelin concentrations from baseline to post-intervention. Additionally, regression analysis revealed no baseline body weight, body composition, and VO_2 peak were predictors of post-intervention fasting ghrelin concentration; nor were any change values in these variables correlated with post-intervention fasting ghrelin.

Discussion

To our knowledge this is the first study to determine the effect of exercise dose on changes in ghrelin concentration in normal weight older women. We found that fasting ghrelin significantly decreased in the higher-dose exercise group but not in the lower-dose group.

These results are in line with our hypothesis, which was there will be different changes in ghrelin concentration in women participating in exercise of two different doses. The participants in the higher-dose group exercised at moderate-intensity for an average of 160 minutes per week and the lower-dose group exercise for 105 minutes per week. Our results therefore indicate non-obese postmenopausal women who meet the public health recommendations for 150 minutes or more of moderate intensity aerobic exercise per week may experience a significant decrease in fasting ghrelin concentrations; while women who are participating in moderate intensity aerobic exercise but are not meeting the recommendation of 150 minutes per week may not experience the changes.

Decreased fasting ghrelin after exercise training has been reported in previous studies in adolescents and mice (13, 14). Prado et al examined the effect of low and high intensity aerobic exercise training on appetite regulating hormones. The participants were obese adolescents, randomized into high or low intensity training groups for 12 weeks. The high intensity group exercised at an intensity that corresponded with their respective ventilator threshold, and the low intensity group exercised at an intensity approximately 20% below their respective ventilator thresholds. Exercise sessions were isoenergetic, with energy expenditure set at 350 kcal per session. The results of this study were intriguing, as there was a decrease in ghrelin concentration in both exercise groups compared to baseline levels, as well as a decrease in body mass in both groups (13). Research by Wang et al in Sprague-Dawly rats found decreased hypothalamic ghrelin concentrations in obese rats subjected to long-term exercise which included 40 minutes of running, 5 days per week, for 8 weeks. Results were compared to long-term controls. Following 8 weeks of exercise, the long-term exercise rats had a decrease in body weight when compared to the long-term controls. In this study, the researchers concluded it was the decrease in hypothalamic ghrelin concentration that ultimately led to the decrease in body weight (14). There are a multitude of potential theories as to why despite weight loss, fasting ghrelin concentrations decreased. These theories are discussed below.

Redistribution of blood flow is a commonly proposed mechanism of action. During exercise, blood is redirected away from the splanchnic area to

keep up with the increased need of working skeletal muscle; being reduced up to 80% during high intensity exercise (15). Because ghrelin is secreted from the stomach, the reduced blood flow to this area may offer an explanation as to why reduced ghrelin levels are seen post-exercise; however this is likely an acute effect. Adiposity signals such as leptin and insulin also appear to play a role. There is an inverse reciprocal relationship between leptin and ghrelin; with research revealing a direct inhibitory effect of leptin on the production of ghrelin (16). Therefore it is possible that alterations in circulating ghrelin concentration may be a secondary change due to changes in body weight.

Lending interest to the theory of a threshold existing, where an individual would have to lose a certain amount of weight before physiological changes such as changes in ghrelin concentration would occur, is a study done by Mason et al, in overweight and obese postmenopausal women. The study was 12 months in duration, and one intervention group was assigned to a diet only intervention. At the conclusion of the 12 months, the diet only group had a non-significant increase in ghrelin of +87 pg/ml compared to baseline levels, or an increase of 6.5%. Upon further examination, the researchers found increases in ghrelin concentration were significantly associated with larger amounts of weight loss. Among women who lost 5-10% of their baseline body weight, ghrelin increased by an average of 60pg/ml compared to baseline levels; and in women who lost 10% or more of their baseline body weight, there was an average increase 148 pg/ml, or a 10.7% increase (7).

Ravussin et al were the first to report the impact of exercise training on circulating ghrelin in a cohort of healthy, young men (12). Following 93 days of a cycling intervention, where the participants performed two bouts of cycling exercise per day to expend 1000 kcal per day, resulting in a 6% body weight loss, there was a 26% increase in fasting ghrelin concentration (12). Another study by Leidy et al furthers this theory. In this study, the participants in this study performed moderate intensity aerobic exercise 5 times per week for a duration that expended 2092 kJ per session (500 kcal). In this particular study, the diet of the participants was tightly controlled, with all participant meals being provided by the research study team. The results found exercise without significant weight loss (<1.5kg) resulted in no changes in fasting plasma ghrelin concentration. However, in participants who experienced a significant amount of weight loss (>1.5kg) fasting ghrelin concentration increased twofold (17). The findings of Leidy et al have been extended to postmenopausal overweight and obese women by Foster-Schubert et al, who found increased concentrations of fasting plasma ghrelin only in the participants who lost weight following a 12 month aerobic exercise intervention of moderate intensity. Their results indicate ghrelin concentrations increased in a “step-wise” fashion, with larger changes occurring in the participants who lost larger amounts of weight (18). These results have been further supported by others who have reported changes in ghrelin levels positively correlated with the extent of weight loss experienced by the participants (8) and another study reporting unchanged fasting plasma ghrelin concentrations following 12 weeks of moderate intensity aerobic exercise

training 5 times per week, in healthy normal weight women who did not lose weight (19). All of the aforementioned studies were done in either males (12), healthy, normal weight young women (17), overweight and obese middle-aged women (8), or postmenopausal overweight and obese women (18). Our study was conducted in non-obese (BMI 18-30 kg/m^2) postmenopausal women, which appear to be a specific segment of the population which had yet to be investigated in this capacity. Previous studies have also focused on an "exercise vs. control" approach, whereas this study sought to compare two different doses of exercise, as it may not be the exercise itself, but the resulting weight loss which then stimulates the change in ghrelin concentration.

When measuring a hormone that is known to fluctuate over the course of the day such as ghrelin, one should take into consideration not only the possible effects of the time of day, but also the potential cumulative effects of energy intake. To have a clear picture of intervention effects on ghrelin, it would be ideal to measure both fasting and postprandial concentrations. Ghrelin concentration is at its lowest levels around approximately 6:00am, and displays meal related increases and decreases throughout the day; with nocturnal peak levels occurring between 1:00am and 2:00am. It has also been shown that acute changes in ghrelin concentration over a normal day of eating behavior in normal weight sedentary women is significantly correlated with calorie intake (17). For this study only collected fasting blood samples were collected, and therefore we are not able to examine if there is any change in postprandial concentrations or the difference between high and low concentrations within a day. The

participants were also free living, so the researchers had no control over the diet being consumed by the participants in terms of macronutrient breakdown or total calorie intake. Also, the duration between blood draw and the last exercise session varies from one to several days, and it is unknown how long any effects of acute or chronic exercise lasts. Therefore, we cannot differentiate whether any effect is due to the last exercise session or exercise training. Additionally, participants were not assessed for feelings of hunger or appetite at any point during the study; therefore, we cannot determine whether the ghrelin response to exercise training is associated with appetite change. Lastly, these results only apply to older non-obese healthy women since ghrelin response to exercise training may be influenced by age, sex, and body weight status. The change in ghrelin concentration in the high dose group corresponded to a medium effect size of 0.56 (Cohen's d); we had 75% power to detect such a change with n=24. The change in ghrelin in the low dose group corresponded to a less than small effect size of 0.16.

All exercise sessions being monitored by trained study staff was a major strength of the study, ensuring all of the exercise sessions were indeed occurring at the correct intensity and meeting calorie expenditure goals. The inclusion of two different doses of exercise allowed us to examine the effect of exercise dose, and if there may be a certain energy expenditure threshold that has to be met in order to illicit changes in ghrelin concentration. Finally, the inclusion of non-obese women allowed us to examine the ghrelin response in individuals with

relatively normal metabolism rather than obese individuals who may already present abnormal metabolic responses.

In conclusion, this study found that fasting ghrelin decreased in older women who participated moderate-intensity exercise of an average of 160 minutes but not in those who exercised at a lower-dose. These results suggest that meeting the public health physical activity guideline may induce decreases in ghrelin responses. However, whether this change is linked to decreased appetite and subsequent weight maintenance worth further investigation.

Future Directions

Future research examining the ghrelin response to long term aerobic exercise should explore the role that the ghrelin receptors play regarding the physiological ghrelin response to exercise; as not only the circulating concentration of the hormone of interest is important, but also the sensitivity, number, and affinity of the respective receptors. Recent research has also indicated an anabolic effect of ghrelin (20) which should also be further examined in respect to chronic aerobic exercise training. This research would also be more suitable for detecting changes in ghrelin receptors in skeletal muscle or body fat, by way of muscle biopsies. Additional research is also needed to further define the physiological mechanisms in regards to changes in circulating ghrelin concentration in response to different exercise interventions.

Table 1.1: Baseline characteristics of participants by exercise groups

	Lower Dose	Higher Dose	p value
Age (years)	65.5 ± 4.6	64.9 ± 4.2	0.61
Height (cm)	161.9 ± 6.7	163.6 ± 6.2	0.35
Weight (kg)	67.3 ± 10.8	68 ± 9.4	0.81
BMI kg/m^2	25.8 ± 4.2	25.5 ± 4.2	0.79
FM (kg)	26.8 ± 7.9	26.8 ± 7.9	0.966
FFM (kg)	41.5 ± 4.2	42.3 ± 5.3	0.54
% Body Fat	38.3 ± 6.8%	38.3 ± 6.6%	0.99
SBP (mmHg)	127.2 ± 13.1	126.8 ± 9.45	0.99
DBP (mmHg)	74.6 ± 8.7	75.7 ± 6.3	0.54
Ghrelin (pg/ml)	76.2 ± 77.3	56.3 ± 54.4	0.28
VO_2 (ml/kg/min)	20.2 ± 3.5	20.2 ± 3.5	0.85

Data are presented as mean ± SD. P values are for between group comparisons.

Table 1.2: Anthropometric and aerobic fitness change values within group

Variables	Lower Dose		Higher Dose	
	Change	P value	Change	P value
Wt (kg)	2.0 ± 8.1	0.79	1.2 ± 2.3	0.65
FM (kg)	0.6 ± 1.7	0.77	1.3 ± 2.2	0.18
LM (kg)	0.1 ± 0.8	0.91	0.2 ± 1.2	0.84
% Body Fat	0.5 ± 1.4	0.79	1.1 ± 1.9	0.57
VO_2 (ml/kg/min)	-0.8 ± 2.8	0.38	-3.0 ± 3.1*	0.02

Data are presented as mean ± SD. P values are paired t-tests within each exercise group. Change values were calculated by subtracting post-intervention values from baseline values. * signifies statistical significance $p < 0.05$

Table 1.3: Ghrelin change values within group

Ghrelin	Lower-Dose	Higher-Dose
Baseline pg/ml	77.2 ± 77.3	63.5 ± 70.7
Post-Intervention pg/ml	56.2 ± 54.4	28.8 ± 14.8
∆ pg/ml	12.7 ± 93.3	27.4 ± 54.8
P value	0.51	0.02 *

Data are presented as mean ± SD. P values are for paired t-tests within each group. * signifies statistical significance

Figure 1.1: Higher-Dose Exercise Group Baseline to Post-Intervention Ghrelin Concentration

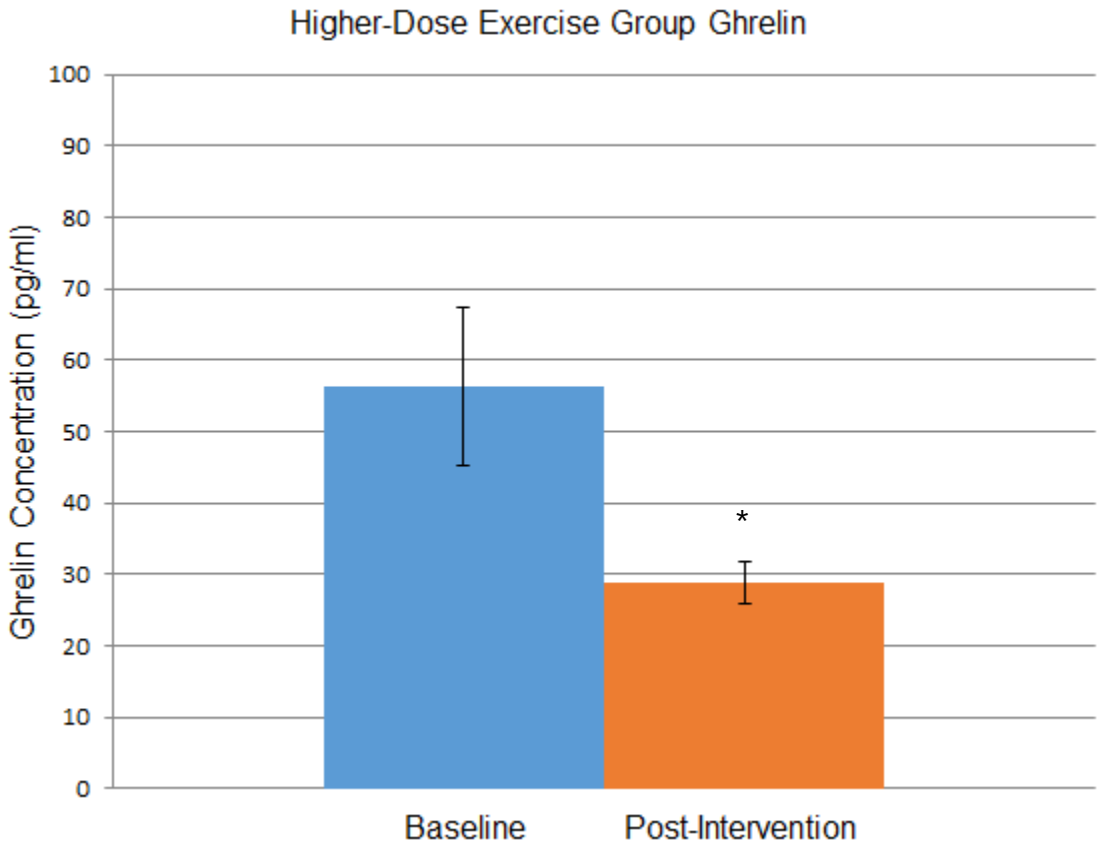

Figure 1.1 shows baseline and post-intervention fasting ghrelin concentration for each participant in the higher-dose exercise group (n=24). Data are presented as mean ± standard error. * signifies statistical significance $p < 0.05$

Figure 1.2: Lower-Dose Exercise Group Baseline to Post-Intervention Ghrelin Concentration

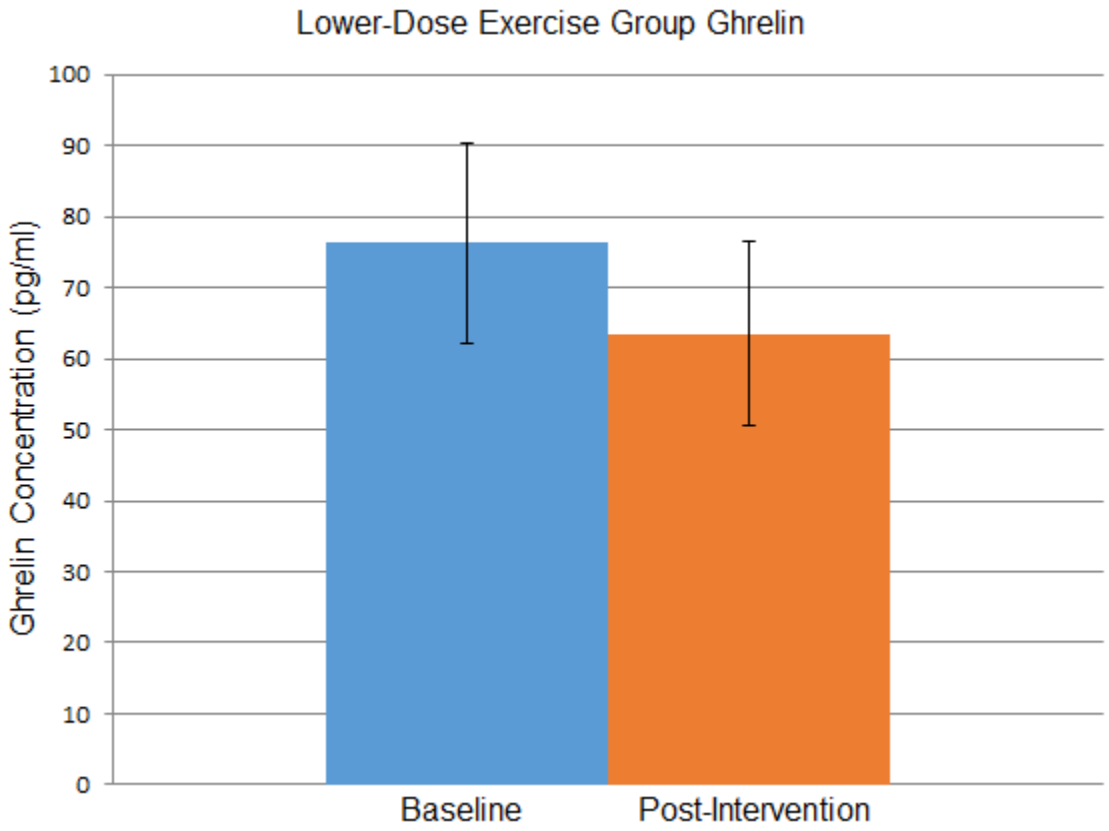

Figure 1.2 shows baseline and post-intervention fasting ghrelin concentration for each participant in the higher-dose exercise group (n=30). Data are presented as mean ± standard error.

CHAPTER 2

THESIS PROPOSAL

Obesity is a global health problem that is associated with increased risks for several diseases such as type 2 diabetes and cardiovascular diseases. In recent years, the prevalence of overweight and obesity in middle aged and older women has continued to increase (1). By 2025, the North American Menopause Society has determined the population of older adults is estimated to increase considerably, and those that are overweight or obese will more than likely need additional clinical care; further extending the cost of healthcare related to obesity. With this in mind, it is evident that this population requires additional attention in regards to understanding and controlling this trend (1).

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(3). Total plasma ghrelin concentrations increase pre-prandially and fall post-prandially (4); however, the post-prandial decrease in ghrelin concentration is absent in obese populations (5). Ghrelin concentrations are found in circulation to be inversely proportional to body weight – higher circulating ghrelin concentrations are found in lower body weights, and lower circulating levels found in higher body weights (5, 7, 8). Following weight change, ghrelin typically responds in a compensatory fashion, with concentrations increasing with weight loss, and decreasing with weight gain (7). Studies of long term caloric restriction ranging from three to six months resulting in significant weight loss have resulted in increased ghrelin concentrations (5, 9).

Exercise as a means to induce negative energy balance and potentially weight loss has been suggested to increase fasting ghrelin concentrations and therefore appetite sensations. There is evidence suggesting that the effect of exercise on ghrelin may be different from caloric restriction induced weight loss (4). Several previous studies have investigated the response of ghrelin concentrations to acute exercise bout and chronic exercise training; however, results of the studies are inconclusive (4, 10). The different dose of exercise used in the studies may be a factor contributing to the inconsistent findings. Additionally, obese individuals may have different responses to exercise intervention (11). Most previous exercise training studies, however, have examined ghrelin response in obese individuals. To our knowledge, normal weight older women are a segment of the population that have yet to be studied in this capacity.

Purpose

Therefore, the purpose of this study is to investigate the ghrelin response to two different doses of chronic exercise training in non-obese older women without a dietary intervention. Data are from the We Walk study (clinicaltrial.gov identifier: NCT01722136), in which older women participated in either a lower- or higher-dose of aerobic exercise training of 4 months; where the higher dose group will meet the current public health guidelines for adults regarding the amount of moderate intensity aerobic exercise per week, while the lower dose group will not. The specific aims of this thesis are outlined below.

Specific Aim 1: To determine whether aerobic exercise training induced changes in ghrelin concentrations.

Specific Aim 2: To determine whether the dose of aerobic exercise training influenced the changes in ghrelin concentrations.

Specific Aim 3: To determine whether the changes in ghrelin concentrations were associated with changes in body weight and body composition.

Hypothesis

Existing literature in this field indicates that following aerobic exercise training programs, overweight to obese postmenopausal women see an increase in circulating ghrelin concentrations with weight loss. Due to our study population consisting of non-obese postmenopausal women, we hypothesize there will be different changes in fasting ghrelin concentration in women participating in two different doses of exercise.

Methods

The study for this thesis used data collected in the Women's Energy Expenditure in Walking Programs (WeWalk) Study (ClinicalTrials.gov identifier: NCT01722136). The WeWalk Study was a 4-month randomized control trial to investigate the effects of moderate intensity exercise of two different doses on energy expenditure in inactive older women. Study procedures were reviewed and approved by the University of South Carolina Institutional Review Board in Columbia, South Carolina. All participants were provided and signed a written informed consent.

Participants and Enrollment Process

The participants of the WeWalk study were non-obese (body mass index, BMI, 18 - 30 kg/m2) older women (60 – 75 years) from the Columbia metropolitan area. They were recruited through advertisements in the local newspapers, email lists, and lunch n' learn programs. Exclusionary criteria included: taking beta blocker medication, metabolic disorders such as diabetes or thyroid disorders, presence of heart diseases, kidney disease, lung disease, cancer diagnoses within the last 5 years (except skin cancer), large weight fluctuations in the last 3 months (> 3%), currently smoking, current participation in another interventional study, or any contraindications to exercise.

This thesis includes a subsample of participants from the WeWalk Study who had both baseline and post-intervention blood samples available for analysis. The total sample size is 54, with 30 in the lower-dose group and 24 in the higher-dose group.

Graded exercise test

Participants completed a maximal graded treadmill test at baseline and post-intervention. The test was an incremental protocol, participants walked at a self-selected pace which remained constant throughout the duration of the test. Every 2 minutes, the incline of the treadmill was increased by 2%. Volume of O2 (VO_2) and CO_2 (VCO_2) was measured via a metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT, USA). Heart rate via a standard 12-lead ECG (Q-Stress; Cardiac Science, Bothell, WA, USA) was monitored by a trained medical personnel. Blood pressure readings were taken approximately 30 seconds into each stage of the test. For the test to be considered satisfactory, two of four criteria had to be met: a plateau in heart rate or VO_2; respiratory exchange ratio greater than or equal to 1.10; a maximum heart rate greater than 90% of age-predicted maximal heart rate (= 220-age); or a rating of perceived exertion greater than or equal to 17 on the Borg scale. VO_2peak was determined by the maximal 30-second averaged VO_2 value sustained during the test.

Exercise Intervention

Participants were randomized into one of two moderate-intensity exercise groups. The lower dose group had an energy expenditure goal of 8 kilocalories (kcal) per kilogram (kg) of body weight per week, and the higher dose group had an energy expenditure goal of 14 kcal/kg of body weight per week. All training sessions were supervised and occurred at the Clinical Exercise Research Center at the University of South Carolina. Both groups exercised for 16 weeks at an intensity of 50-55% of the participants heart rate reserve (HRR), which was

calculated using information obtained from each woman's graded exercise test. Due to the participants being inactive prior to their participation in the study, training intensity started at 40% HRR and was increased by 5% every two weeks until the target level of 50-55% was achieved. The exercise target was reached within 5 weeks for women in the low dose group and 8 weeks for those in the high dose group. Heart rate monitors (FT1; Polar; Lake Success, NY, USA) were worn during training sessions to ensure participants were exercising at the desired intensity throughout each exercise session. Mid-way through the 16 weeks of exercise training, participants completed a submaximal VO_2 test to ensure they were exercising at the correct intensity given the exercise training group they were randomized into. Heart rate was monitored and recorded every 5 minutes throughout each training session, and blood pressure was measured before, at the midpoint of the exercise session, and after the exercise sessions.

Body Composition

The dual-energy absorptiometry (enCORE, GE Healthcare model 8743, Waukesha, WI) full body scan was utilized to measure body fat percentage, lean mass (kg), fat mass (kg) and fat free mass (kg) at baseline and post-intervention. Height was measured to the nearest 0.1cm without shoes, and weight was measured to the nearest 0.1kg while the participant was wearing lightweight scrubs. Two measurements for both height and weight were taken, and averages were used to calculate BMI (kg/m2).

Blood Sample Collection and Analysis

Venous blood samples were collected at baseline and following the exercise intervention. Participants were instructed to fast (not including water) for at least 12 hours prior to blood draw visits. Blood samples were drawn into EDTA tubes, centrifuged at 3,000 rpm for 20 minutes at 4 degrees Celsius. Plasma was then aliquoted into Eppendorf tubes and stored at -80 degrees Celsius until analysis.

Plasma samples were thawed and re-centrifuged at 3,000 rpm at 4C° for 20 minutes to remove any potential particulates before analysis of ghrelin concentration. Plasma ghrelin was quantified by a multiplex immunoassay (Human Metabolic Hormone Magnetic Bead Panel, Millipore, Billerica, MA) per manufacturer instructions on a Magpix multiplexing system (Luminex, Austin, Texas, USA). The assay has a minimum detectable concentration of 13 pg/ml. The intra-assay coefficient of variance (CV) is < 10% and inter-assay CV is < 15%. All samples were run in duplicates.

Statistical Analysis

Descriptive statistics will be first examined. Data will be reported as mean ± SD for normally distributed variables and median (quartiles) for non-normally distributed variables, or frequencies in percentages. Change values in body weight, fat mass, fat-free mass, and ghrelin concentration will be calculated using values after intervention minus at baseline. Baseline values will be compared between the exercise groups using one-way analysis of variance (ANOVA) for normally distributed variables, Kruskal-Wallis test for non-normally distributed

continuous variables, and chi-square test for categorical variables. ANOVA with repeated measures including an interaction term (group by time) will be used to determine if the change after exercise training in body weight, fat mass, fat-free mass, and ghrelin concentration is different between the two exercise groups. If necessary, data will be transformed to achieve normal distribution prior to performing ANOVA. If the group by time interaction is significant, ANOVA with repeated measures will be performed in each group separately. Because of the reported association between body weight and body composition with ghrelin concentration in the literature, baseline and changes in body weight, fat mass, and fat-free mass will be adjusted for as covariates in the ANOVA. Pearson's correlation between body weight and body composition and ghrelin concentration will be calculated for baseline values and change values from baseline to post-intervention. A p value < 0.05 will be used to denote statistical significance. The SAS software (version 9.4, SAS Institute, Cary, NC) will be used to perform analyses. Additionally, the power will be calculated with the achieved effect size, in order to inform future studies.

Strengths and Limitations

All exercise sessions being monitored by trained study staff is a strength of the study, as we can say with confidence that the participants were in fact exercising at the correct intensity as well as meeting the energy expenditure goals of the group they were assigned to. No exercise sessions for any participant were done outside of the Clinical Exercise Research Center at USC. The inclusion of two doses will allow us to examine the effect of exercise dose.

The higher-dose exercise group exercised for an average of 160 minutes per week, which is just above the current public health recommendations for adults with respect to moderate intensity aerobic exercise. This allows us to examine the ghrelin response in non-obese postmenopausal women meeting the guidelines. The lower-dose exercise group exercised on average for 105 minutes per week, which falls below the 150 minutes per week of moderate intensity aerobic exercise currently recommended for adults by the Centers for Disease Control. The inclusion of non-obese women will allow us to examine the response in individuals with relatively normal metabolism rather than obese individuals who may already present abnormal metabolic responses. On the other hand, the results only apply to older non-obese healthy women since ghrelin response to exercise training may be influenced by age, sex, and body weight status. We have only collected fasting blood samples therefore we are not able to examine if there is any change in postprandial concentrations or the difference between high and low concentrations within a day. Also, the duration between blood draw and the last exercise session varies from one to several days, and it is unknown how long any effects of acute or chronic exercise lasts. Therefore, we cannot differentiate whether any effect is due to the last exercise session or exercise training. Additionally, participants were not assessed for feelings of hunger or appetite at any point during the study; therefore, we cannot determine whether the ghrelin response to exercise training is associated with appetite change.

Literature Review

This section will review the physiology and function of ghrelin, how ghrelin relates to energy intake and body weight, the acute effects of exercise on ghrelin concentration, how chronic aerobic exercise training affects ghrelin, exercise training and body weight and body composition, as well as the relevant literature regarding ghrelin in animal models.

Physiology and Function of Ghrelin

Ghrelin is a 28 amino acid peptide secreted from the stomach and the only known circulating orexigenic hormone, discovered by Kojima et al in 1999 (21). Despite being a primarily peripherally secreted peptide, ghrelin exerts its major effects on the central nervous system, playing a role in the regulation of energy balance by increasing appetite and initiating food intake, as well as reducing fat utilization (3). The first report of ghrelin's involvement in appetite and the regulation of energy balance surfaced in a study focusing on growth hormone secretion, done by Arvat et al, where hunger was reported as a "collateral effect" of ghrelin administration (22). Though mostly produced by the P/D1 cells in the fundus of the stomach, there is also evidence that some ghrelin is produced in the ARC region of the hypothalamus, though its significance is still unknown (23).

Ghrelin is acylated into its active form by ghrelin a-acyl-transferase, or GOAT (23). The acyated form of ghrelin has a half-life of approximately 10 minutes, where the deacylated form has a half-life about threefold longer (24). The acylated form of ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHSR), and is believed to be the form of ghrelin

responsible for controlling appetite and energy homeostasis (25). The GHSR is expressed throughout the hypothalamus, and the effects of ghrelin are mediated by the appetite stimulating neurons in the arcuate nucleus containing neuropeptide Y and agouti-regulated peptide (NPY/AgRP) (26). Though ghrelin circulates in two forms, acylated and desacylated, research has shown that the ratio of acylated ghrelin to total ghrelin remains consistent under most conditions (11).

Many studies have investigated the effect of either an IV bolus or a continuous infusion of exogenous ghrelin. These studies, however, have utilized fairly high doses of ghrelin, therefore resulting in significantly supraphysiological concentrations. Studies employing IV bolus application of ghrelin have used varying does between 40 micrograms (27) and 3 micrograms/kg body weight, resulting in plasma ghrelin concentrations of approximately 5,000 pg/ml (28-30). Continuous ghrelin infusion studies have used doses varying between 3.37 nanograms/kg body weight/per minute to 50.7 nanograms/kg/min with infusion period lasting anywhere from 75 minutes to 360 minutes (31-35). The total ghrelin levels witnessed at the lowest doses of continuous infusion protocols fell between a range of 2,500 and 5,000 picograms/ml. More recent research of ghrelin infusion by Lippl et al utilized a low-dose ghrelin infusion method of 1 ng/kg/min, mimicking a near-physiological dose of ghrelin, or a saline placebo. Participants were healthy, young males. In this study, the ghrelin infusion was started an inter-meal time period, which they argue is more appropriate physiological approach for examining ghrelin as the nadir of endogenous ghrelin

secretion occurs following a meal. The results from this study indicate an infusion of near-physiological levels of ghrelin did not have an effect on meal size, meal timing, or hunger stimulation (36).

Although it appears supraphysiological doses of ghrelin administered via infusion are necessary to induce an appetite response, there may be other clinical applications of ghrelin, particularly in clinical diagnoses cachexia resulting from an array of pathologies such as chronic heart, renal, pulmonary, and other disease states, or those going through chemotherapy treatment. Research by Akamizu et al sought to determine the clinical response to repeated administration of ghrelin to patients with functional dyspepsia, a chronic peristalsis disorder; and decreased food intake. The participants in this study received an IV ghrelin infusion of 3 micrograms/kg for 30 minutes, twice daily, before breakfast and dinner meals, for two weeks. The results found ghrelin administration in this cohort tended to increase food intake when compared to food intake level before and in the days following treatment (37). Another study of ghrelin infusion in a clinical population investigated the effects of ghrelin administration in cancer patients with impaired appetite. This study was an acute, randomized, placebo controlled cross-over trial where the participants received either a saline control infusion or an infusion of ghrelin at the rate of 5 pmol/kg. When compared to the saline control, there was a marked increase in energy intake during the ghrelin infusion trial of 31.7 ± 7%. These results indicate ghrelin infusion could be an effective treatment for anorexia secondary to other clinical diagnoses (38).

Ghrelin, Energy Intake, & Body Weight

Total plasma ghrelin concentrations increase pre-prandially and fall post-prandially, indicating its role in the initiation of energy intake and short term energy homeostasis (4). Ghrelin suppression following a meal tends to be greater after a larger, high calorie meal; but may also be influenced by the macronutrient breakdown of the meal - carbohydrates being more suppressive than proteins or fats (39). The exact mechanisms of post-meal ghrelin suppression have yet to be fully discovered, but is likely related to the detection of nutrients in the stomach and possibly gastric emptying. The post-prandial decrease in ghrelin concentration is seen in lean subjects, however in obese populations, this suppression is not witnessed, possibly leading to greater energy intake, further perpetuating the obesity issue (11). As ghrelin has been associated with feelings of hunger and regulation of energy intake, its role in weight loss is of great interest. Ghrelin concentrations are found in circulation to be inversely proportional to body weight – higher circulating ghrelin concentrations are found in lower body weights, and lower circulating levels found in higher body weights (5, 7, 8).

Following weight change, ghrelin typically responds in a compensatory fashion, with concentrations increasing with weight loss, and decreasing with weight gain (7). Studies of long term caloric restriction ranging from three to six months resulting in significant weight loss have resulted in increased ghrelin concentrations (5, 9). In addition to ghrelin, it has been shown that weight loss achieved through caloric restriction results in changes in appetite hormones such

as leptin, peptide tyrosine tyrosine (PYY) , and cholecystokinin (CCK); as well as changes in feelings of hunger that persist for 12 months following weight loss (1). These changes in appetite hormones may also improve the sensitivity of the appetite control system (40).

Effects of single exercise bout on ghrelin

The effect of acute bout of aerobic exercise on plasma ghrelin concentrations has been studied, but the findings are inconclusive. Several studies have investigated the effect of a single session of exercise at various intensities and durations on ghrelin and found no changes in its concentration. These studies include a treadmill run for an hour at 73.5% of VO_2max in young, healthy males and females (41), an intermittent treadmill running protocol (60% of VO_2 max for 10 minutes, 75% VO_2 max for 10 minutes, 90% VO_2 max for 5 minutes, and then 100% of VO_2 max for 2 minutes) in young, well trained males (42), acute aerobic exercise at 50% or 70% of VO_2 max for 20 minutes, or 90% VO_2max intermittently for 10 minutes with 30 seconds followed by 30 seconds of rest in young, healthy males (43). All of the exercise bouts in these studies were of either high or moderate intensity, were of short duration, and the participants were young and lean. The findings from these studies indicate a single session of exercise less than 20 minutes in duration have no effect on ghrelin concentrations upon the completion of exercise regardless of intensity in healthy, young populations.

Erdmann et al. designed a study to investigate both the effect of exercise duration and the effect of exercise intensity on changes in plasma ghrelin

concentration (44). The intensity trial in this study varied slightly from the studies discussed above, as the exercise was 30 minutes in duration, and was done on a cycle ergometer as opposed to treadmill running. Participants were young adults of normal weight. The experimental trials for the intensity group had the participants cycle for 30 minutes at the low intensity of 50 W, and the high intensity trial was 100W. In response to the 50W trial, ghrelin concentrations significantly rose from an average baseline value of 520.4 ± 82.7 pg/ml to 566.6 ± 86.2 pg/ml following the exercise compared to a resting control. There were no significant changes in ghrelin concentration during the 100W trial. The experimental trials for exercise duration had the participants cycle at 50W for 30, 60, and 120 minutes. Ghrelin concentrations rose significantly 50-70 pg/ml during the respective period of exercise for the duration trial, but were not significantly different following any of the exercise trials. These results indicate that low intensity exercise 30 minutes or longer may bring about changes in ghrelin concentration independent of duration.

Additionally, other researchers have investigated the plasma ghrelin concentration response to an energy deficit either created by an acute exercise session, or achieved through a caloric deficit. King et al compared a single exercise bout of a 90-minute run at 70% VO_2max and an equal energy deficit created by calorie restriction from 2-4.75 hour (0 hour was defined as the time corresponding to immediately finishing exercise), and a control trial in healthy, lean, young adult males (4). Across trials, plasma acylated ghrelin was significantly higher in the caloric restriction trial when compared to the control

and exercise trials at time points 2, 3, 4.75, 6, 7, and 8 hours. There were no significant differences in ghrelin concentration at any of the time points between the control and exercise trials. Another study in normal weight males with a similar design showed different results. The exercise was 30 minutes of moderate-intensity (65% of VO_2max) cycling (10). The caloric restriction was equivalent to the energy deficit in the exercise trial. Their results found the area under the curve for acylated ghrelin concentration tended to be higher in the control trial than in the exercise deficit or calorie deficit trials from 0 to 1 hour, but did not differ between trials at any other time period. An increase in appetite, however, was found after caloric restriction, but not when the energy deficit was achieved via an acute bout of exercise. These changes in appetite were unrelated to changes in acylated ghrelin concentration. Although these two studies showed different results regarding ghrelin changes after caloric restriction, the collective results indicate effects of exercise on ghrelin may not be completely dependent on energy balance.

Further, another study found treadmill walking at 55-60% of VO_2 max for an hour in the evening induced an 18% lower acylated ghrelin concentration in the next morning compared to no-exercise control trial in normal weight individuals (11). Interestingly, in this study, obese participants did not show a change in ghrelin following the same exercise protocol, although lower levels of satiety following the exercise trial were reported when compared to the no-exercise control trial. The authors concluded that exercise suppressed ghrelin

concentration in normal weight individuals, but this response in ghrelin to exercise was blunted in obese individuals.

In summary, the intensity and duration of a single bout of exercise appear to influence the changes in ghrelin concentration. An exercise bout longer than 30 minutes at low to moderate-intensities may induce changes in ghrelin; however, one study showed increases (44) while another one showed reductions (11). Normal weight and obese individuals may have different responses to exercise. Also, exercise's effects on ghrelin may not be completely dependent on the amount of energy deficit, since isocaloric deficits created by exercise or caloric restriction induces different changes in ghrelin and appetite sensation.

Effects of chronic exercise training on ghrelin

Fewer studies have investigated the effect of exercise training on ghrelin concentration. The inconsistent results are likely related to the various intensity and duration of the exercise sessions, length of training, and the characteristics of the participants.

A 5-day exercise training of treadmill running in healthy, young males and females (45 minutes at 55% maximum heart rate reserve each day) did not result in significant changes in the concentration of acylated ghrelin compared to the no-exercise control (45). An exercise training of 12 weeks also found no change in ghrelin concentration. In this study, middle-aged overweight or obese men completing 60 minutes per session of aerobic training at 75-80% of their heart rate maximum for 3 sessions per week had a significant decline in body mass of 2.0 kg, but had no change in fasting ghrelin concentration (46).

Similarly, another study of long duration (12 months) in overweight and obese postmenopausal women showed no change in fasting ghrelin concentration after exercise training (7). The exercise was 45 minutes of moderate to intense aerobic exercise five days per week; there was no significant change in ghrelin concentration, though the average weight loss was -2.4%. Among women who lost 5-10% of their starting body weight, the mean ghrelin concentration had a nonsignificant increase of 2.2%, and too few women lost more than 10% of their body weight in the exercise only group for a valid analysis. In the same study, a dietary restriction intervention resulted in women losing 5-10% of their starting body weight having an increase in ghrelin concentration of 4.2%, and those that lost more than 10% of their starting body weight had an increase in ghrelin concentration of 10.7%. They concluded that greater amounts of weight loss were associated with non-significantly greater increases in ghrelin concentrations. However, similar amounts of weight lost by diet compared to exercise alone seems to cause a greater increase in ghrelin concentration.

In contrast, in another study, overweight, postmenopausal women experienced a significant increase of plasma ghrelin of +32 ± 16 pg/mL from a baseline of 599 ± 16 pg/mL after 12 months of aerobic exercise training (18). The aerobic exercise was 45 minutes and 5 days a week at 60-75% of their maximal heart rate. There was a small but significant weight loss of 1.4kg ($p < 0.05$).

The above studies in adults show that ghrelin either does not change or increases following aerobic exercise training. One study in obese adolescents found contradictory results (13). In this study, adolescent participants were randomized into either high intensity or low intensity exercise training, and received the same nutritional, psychological and clinical counseling throughout 12 weeks of the study. The high intensity group exercised at an intensity that matched their ventilatory threshold, and the low intensity group exercised at an intensity approximately 20% lower than their respective ventilatory threshold. The exercise sessions were isoenergetic, where energy expenditure was set at 350 kcal, and for three times a week. Upon completion of the study, both exercise groups showed a reduction in ghrelin levels. These results are intriguing as both groups saw a decrease in body weight; which corresponds to either an unchanged or an increased circulating ghrelin level in the studies performed in adults discussed above. Thus, age of participants may be a factor affecting the response of ghrelin to exercise training.

Additionally, two other studies examined postprandial ghrelin response following exercise training. In one study, 12 non-obese, young sedentary women were randomized into non-exercising control group or a diet and exercise energy deficit group for three months. In the energy deficit group, exercise was done 5 day a week at 70-80% of VO_2max as determined by a maximal treadmill test; and there was an average weight loss of 2.5kg ± 0.9, as well as a significant increase in the 24 hour area under the curve of ghrelin. There were also significant changes in ghrelin including elevations in baseline from a pre-intervention

concentration of 1411 ± 107 pg/mL to a post-intervention concentration of 1764 ± 113 pg/mL; lunch peak pre-intervention concentration of 1670 ± 121 pg/mL to post-intervention concentration of 2040 ± 105 pg/mL; dinner peak from pre-concentration concentration of 1977 ± 123 pg/mL to post-intervention concentration of 2414 ± 134 pg/mL; and nocturnal peak pre-intervention concentration of 1862 ± 96 pg/mL to post-intervention concentration of 2372 ± 148 pg/mL (17). In another study, sedentary, middle-aged, overweight or obese individuals (n=22) completed 12 weeks of moderate intensity exercise 5 times per week at 75% of their maximal heart rate (40). The intervention resulted in a significant reduction in body weight (3.5 kg). Their results yielded a significant increase in fasting acylated ghrelin following the exercise intervention from a baseline concentration of 37.3 ± 18.2 pmol/L to a post intervention concentration of 51.7 ± 26.0 pmol/L, as well as a significant increase in the suppression of acylated ghrelin postprandially. These findings point to increased appetite sensations following exercise-induced weight loss; this change is also accompanied by a greater sensation of satiety to energy intake.

In summary, previous studies of chronic exercise show inconsistent findings regarding fasting ghrelin concentration changes. One study showed that with similar weight loss, ghrelin responses to exercise and caloric restriction were different (21), suggesting the exercise effect on ghrelin is not completely dependent on weight change. This is also supported by other studies that a small weight loss resulted in increased fasting ghrelin (43), but greater weight loss did not result in weight loss in another study (21). Additionally, age of participants or

the length of being obese may affect ghrelin response (11),(47). Further, the ghrelin changes in response to exercise training is also shown in the amplitude of change during a day.

Exercise Effects on Body Weight and Composition

Previous studies have examined the effects of aerobic exercise training alone on body weight and body composition. In this section, several large scale exercise trials are reviewed.

One study of 400 inactive older women with a BMI between 22–40 kg/m2 compared the effects of 300 minutes per week of moderate to vigorous intensity exercise to 150 minutes per week (48). The results found high volume (300 minutes per week) was better than moderate volume (150 minutes per week) in regards to reducing total body fat. The high volume exercise group experienced an average body fat loss of -2.2%, while the moderate volume group experienced an average body fat loss of -1.2%. There was also a dose-response relationship for decreases in BMI, waist circumference measurements, and waist to hip ratio.

The Studies of Targeted Risk Reduction Intervention through Defines Exercise (STRRIDE) study consisted of an eight month exercise program with participants randomized into one of three groups: high amount with vigorous intensity, equivalent of 20 miles per week at 65-80% VO_2max, low amount with vigorous intensity, equivalent of 12 miles per week at 65-80% VO_2max, and low amount of moderate intensity, equivalent of 12 miles per week at 40-55% VO_2max (49). The results indicate that in overweight participants, both of the low amount groups lost body weight and fat, and the high amount group lost

more body weight and body fat compared to the low amount groups; all in the absence of caloric restriction. There was a clear dose response relationship between the amount of exercise done per week and weight loss: the low amount moderate intensity group lost an average of approximately 2kg, the low amount vigorous intensity group lost approximately 2.5kg, and the high amount vigorous intensity group lost the most weight at approximately 5kg. These findings further solidify exercise can be an effective approach to losing both body weight and fat in overweight adults.

The Midwest Exercise Trial set out to investigate the efficacy of a known amount of exercise to prevent weight gain or to produce weight loss in overweight and moderately obese adults (50). Participants randomized to the exercise group exercised at 75% heart rate reserve (HRR) for 45 minutes, five days per week. The targeted energy expenditure of each exercise session was approximately 400 kilocalories. The control group was instructed to maintain their usual physical activity and dietary habits. The results found that exercise had a significant effect on body weight and composition; however the effects were different between men and women. Men in the exercise group lost an average of 5.2 kilograms of body weight, which was approximately -6% of baseline weight. Almost all (96%) of the weight loss came from body fat, and visceral fat decreased by 23%. Women assigned to the exercise group did not experience weight loss; however they did avoid the 3 kg weight gain that was seen in the women in the control group.

The Dose Response in Exercise in Women (DREW) study was a randomized dose-response exercise trial in pre-hypertensive obese postmenopausal women. There were 3 exercise groups with incrementally higher doses of energy expenditure and a non-exercise control group (51). The three exercise groups were classified by energy expenditure as follows: 4 kcal/kg/week, 8 kcal/kg/week, and 12 kcal/kg/week. The women in the exercise groups participated in 3 or 4 training sessions each week, at approximately 50% of their respective VO_2max, for six months. Upon completion of the study, all exercising groups had lost a significant amount of weight: the 4 kcal/kg/week group had lost an average of -1.4 kg, the 8 kcal/kg/week group an average of -2.1 kg, and the 12 kcal/kg/week group an average of -1.5 kg.

The Sex Hormones and Physical Exercise study (SHAPE-2) study was a randomized control trial done in postmenopausal women. The overall design of the study was intended to have participants in the experimental groups lose body weight. One arm was “mainly exercise induced weight loss”, where the participants were assigned to follow an intensive 4 hour per week exercise program consisting of mainly endurance exercise with some added strength training; and a small caloric deficit of approximately 250 kcal/day. The other experimental arm was calorie restriction only. The experimental period lasted for 14 weeks. After the 14 weeks, the “mainly exercise” group accomplished an average weight loss of -5.5kg (6.6%). The results from the SHAPE-2 study indicate losing weight through exercise as opposed to dietary restriction alone

resulted in a larger loss of fat mass and preservation of lean mass, which is an important consideration especially in older populations (52).

In summary, weight loss can be achieved by aerobic exercise training alone, which appears to be up to approximately 7%. There is also an accompanying overall loss of adiposity. The amount and intensity of exercise play a role in the amount of weight and fat loss.

Animal Research on Ghrelin

Numerous studies have utilized animal models to confirm the physiological function of ghrelin and its receptor. Work by Zigman and colleagues confirmed the GHSR as the only receptor responsible for ghrelin's acute orexigenic properties (53). To investigate the physiological significance of GHSR transcriptional blockage on long term body weight maintenance, researchers fed the GHSR-null mice and their wild-type (WT) littermates a Western-type high fat diet (HFD) for 19 weeks. Body composition was determined by multiple methods, including DXA, nuclear magnetic resonance (NMR) and carcass analysis via lyophilization and standard ether extraction. In female mice, after 8 weeks of HFD feeding, the GHSR-null mice weighed significantly less than the WT mice and thereafter continued to increasingly diverge in body weight. After 19 weeks, the GHSR-null mice weighed 12.7% less than their WT counterparts; and had 46.5% less fat mass than the WT mice. In male mice, the GHSR-null mice weighed 10.9% less than their WT counterparts after 6 weeks on HFD, and at completion of the study had 16.5% less fat mass when compared to the WT male mice. The results also indicate GHSR-null mice on HFD are hypophagic. The

GHSR-null mice manifested statistically significantly lower average weekly food intake compared to WT mice. Researchers concluded that ghrelin signaling via GHSR is necessary for the full development of diet induced obesity. The results also suggest a sex difference on fat mass.

Other research with animals has examined the effects of acute and chronic exercise on ghrelin. One study compared acute aerobic exercise to chronic aerobic exercise in rats. In the acute arm, Sprague-Dawley rats were forced to run on a treadmill for 40 minutes; and following the exercise, the rats consumed smaller meals and had decreased hypothalamic ghrelin for the next three hours (14). The arm investigating chronic aerobic exercise had obese rats run on a treadmill 5 days per week for 8 weeks. Compared to the controls, the exercising group lost body weight, consumed smaller meals, and had lower fasting hypothalamic ghrelin concentrations (14). Therefore, both the acute and chronic exercise showed suppressive effects on ghrelin concentrations.

Another study in rats examined the relationship between chronic resistance exercise and ghrelin concentration (54). The rats performed resistance exercise five times per week for five weeks. The results found after the five weeks of training, the rats body mass decreased, and total fasting ghrelin concentration decreased by 20%. This study also supports a suppressive effect of exercise on ghrelin.

Summary

Ghrelin is the only known circulating orexigenic hormone, and given today's obesity epidemic, understanding its response to exercise as a means to

control body weight is critical. It circulates in inverse proportion to body weight, and this relationship is maintained following weight loss. The effect of acute exercise on ghrelin concentration has been studied, and most of those studies included a relatively short exercise bout of 20 minutes or less resulted in no change in ghrelin concentration, regardless of the intensity that the exercise was performed. It should also be noted that these studies were conducted in young, healthy participants. A single exercise bout greater than 30 minutes may induce change in ghrelin concentrations, however one study showed an increase (44) while another showed a decrease (5). Another study has also produced results that may lead us to believe it is possible that normal weight and obese individuals do not have the same ghrelin response to exercise (11). Studies of ghrelin concentration changes following long term exercise training studies in adults have shown that ghrelin concentration either does not change, or increases. In many of these studies, participants were overweight and over the course of exercise training lost weight, though it may not have been a significant amount; and an increase in ghrelin concentration following weight loss is expected. However, a study in adolescents where significant weight loss occurred resulted in a decrease in plasma ghrelin levels, contradicting the response shown in adults (48).

Normal weight older women appear to be one segment of the population that has not been studied previously regarding the ghrelin response to chronic exercise training. As indicated by a study of acute exercise (11), normal weight and obese individuals may have differing responses. Many studies have

investigated the effect of intensity of exercise on changes in ghrelin concentration, however for this study we plan to examine the role that exercise dose potentially plays in changes in ghrelin concentration. This study will be the first, to our knowledge, to investigate changes in ghrelin concentration in older normal weight women without intentional weight loss. In this thesis, ghrelin concentrations before and after aerobic exercise at two different doses will be examined in non-obese older women.

REFERENCES

1. Baker A, Sirois-Leclerc H, Tulloch H. The Impact of Long-Term Physical Activity Interventions for Overweight/Obese Postmenopausal Women on Adiposity Indicators, Physical Capacity, and Mental Health Outcomes: A Systematic Review. Journal of Obesity. 2016;2016:6169890.

2. Hagobian TA, Sharoff CG, Stephens BR, Wade GN, Silva JE, Chipkin SR, et al. Effects of exercise on energy-regulating hormones and appetite in men and women. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2009;296(2):R233-42.

3. Gil-Campos M, Aguilera CM, Canete R, Gil A. Ghrelin: a hormone regulating food intake and energy homeostasis. The British journal of nutrition. 2006;96(2):201-26. Epub 2006/08/23.

4. King JA, Wasse LK, Ewens J, Crystallis K, Emmanuel J, Batterham RL, et al. Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. J Clin Endocrinol Metab. 2011;96(4):1114-21. Epub 2011/01/29.

5. Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, et al. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. European journal of endocrinology. 2001;145(5):669-73. Epub 2001/11/27.

6. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, et al. Ghrelin increases food intake in obese as well as lean subjects. International journal of obesity (2005). 2005;29(9):1130-6. Epub 2005/05/27.

7. Mason C, Xiao L, Imayama I, Duggan CR, Campbell KL, Kong A, et al. The effects of separate and combined dietary weight loss and exercise on fasting ghrelin concentrations in overweight and obese women: a randomized controlled trial. Clinical endocrinology. 2015;82(3):369-76. Epub 2014/05/07.

8. Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, et al. Weight loss increases circulating levels of ghrelin in human obesity. Clinical endocrinology. 2002;56(2):203-6. Epub 2002/03/05.

9. Crujeiras AB, Goyenechea Eb, Abete I, Lage M, Carreira MC, Martínez JA, et al. Weight Regain after a Diet-Induced Loss Is Predicted by Higher Baseline Leptin and Lower Ghrelin Plasma Levels. The Journal of Clinical Endocrinology & Metabolism. 2010;95(11):5037-44.

10. Deighton K, Batterham RL, Stensel DJ. Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. Appetite. 2014;81:52-9. Epub 2014/06/10.

11. Heden TD, Liu Y, Park Y, Dellsperger KC, Kanaley JA. Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. Journal of Applied Physiology. 2013;115(5):680-7.

12. Ravussin E, Tschop M, Morales S, Bouchard C, Heiman ML. Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. J Clin Endocrinol Metab. 2001;86(9):4547-51. Epub 2001/09/11.

13. Prado WL, Lofrano-Prado MC, Oyama LM, Cardel M, Gomes PP, Andrade ML, et al. Effect of a 12-Week Low vs. High Intensity Aerobic Exercise Training on Appetite-Regulating Hormones in Obese Adolescents: A Randomized Exercise Intervention Study. Pediatric exercise science. 2015;27(4):510-7. Epub 2015/12/19.

14. Wang J, Chen C, Wang RY. Influence of short- and long-term treadmill exercises on levels of ghrelin, obestatin and NPY in plasma and brain extraction of obese rats. Endocrine. 2008;33(1):77-83. Epub 2008/04/05.

15. Hazell TJ, Islam H, Townsend LK, Schmale MS, Copeland JL. Effects of exercise intensity on plasma concentrations of appetite-regulating hormones: Potential mechanisms. Appetite. 2016;98:80-8. Epub 2016/01/02.

16. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach. Regulatory peptides. 2004;119(1-2):77-81. Epub 2004/04/20.

17. 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, Md). 2007;15(2):446-55. Epub 2007/02/15.

18. Foster-Schubert KE, McTiernan A, Frayo RS, Schwartz RS, Rajan KB, Yasui Y, et al. Human plasma ghrelin levels increase during a one-year exercise program. J Clin Endocrinol Metab. 2005;90(2):820-5. Epub 2004/12/09.

19. Hill BR, Rolls BJ, Roe LS, De Souza MJ, Williams NI. Ghrelin and peptide YY increase with weight loss during a 12-month intervention to reduce dietary energy density in obese women. Peptides. 2013;49:138-44.

20. Esposito A, Criscitiello C, Gelao L, Pravettoni G, Locatelli M, Minchella I, et al. Mechanisms of anorexia-cachexia syndrome and rational for treatment with selective ghrelin receptor agonist. Cancer treatment reviews. 2015;41(9):793-7. Epub 2015/09/21.

21. 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(6762):656-60.

22. Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, et al. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. Journal of endocrinological investigation. 2000;23(8):493-5. Epub 2000/10/06.

23. Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. Endocrinology. 2010;151(10):4745-55. Epub 2010/09/10.

24. Tong J, Dave N, Mugundu GM, Davis HW, Gaylinn BD, Thorner MO, et al. The pharmacokinetics of acyl, des-acyl, and total ghrelin in healthy human subjects. European journal of endocrinology. 2013;168(6):821-8. Epub 2013/03/14.

25. Delporte C. Structure and physiological actions of ghrelin. Scientifica. 2013;2013:518909. Epub 2014/01/02.

26. Sakata I, Park WM, Walker AK, Piper PK, Chuang JC, Osborne-Lawrence S, et al. Glucose-mediated control of ghrelin release from primary cultures of gastric mucosal cells. American journal of physiology Endocrinology and metabolism. 2012;302(10):E1300-10. Epub 2012/03/15.

27. Ang D, Nicolai H, Vos R, Mimidis K, Akyuz F, Kindt S, et al. Influence of ghrelin on the gastric accommodation reflex and on meal-induced satiety in man. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2009;21(5):528-33, e8-9. Epub 2009/02/13.

28. Adachi S, Takiguchi S, Okada K, Yamamoto K, Yamasaki M, Miyata H, et al. Effects of ghrelin administration after total gastrectomy: a prospective, randomized, placebo-controlled phase II study. Gastroenterology. 2010;138(4):1312-20. Epub 2010/01/12.

29. Schmid DA, Held K, Ising M, Uhr M, Weikel JC, Steiger A. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2005;30(6):1187-92. Epub 2005/02/03.

30. Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. Endocrine journal. 2009;56(9):1119-28. Epub 2009/09/17.

31. Huda MS, Dovey T, Wong SP, English PJ, Halford J, McCulloch P, et al. Ghrelin restores 'lean-type' hunger and energy expenditure profiles in morbidly obese subjects but has no effect on postgastrectomy subjects. International journal of obesity (2005). 2009;33(3):317-25. Epub 2009/02/04.

32. Vestergaard ET, Hansen TK, Gormsen LC, Jakobsen P, Moller N, Christiansen JS, et al. Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects. American journal of physiology Endocrinology and metabolism. 2007;292(6):E1829-36. Epub 2007/02/22.

33. Levin F, Edholm T, Schmidt PT, Gryback P, Jacobsson H, Degerblad M, et al. Ghrelin stimulates gastric emptying and hunger in normal-weight humans. J Clin Endocrinol Metab. 2006;91(9):3296-302. Epub 2006/06/15.

34. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001;86(12):5992. Epub 2001/12/12.

35. Falken Y, Hellstrom PM, Sanger GJ, Dewit O, Dukes G, Gryback P, et al. Actions of prolonged ghrelin infusion on gastrointestinal transit and glucose homeostasis in humans. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2010;22(6):e192-200. Epub 2010/01/27.

36. Lippl F, Erdmann J, Steiger A, Lichter N, Czogalla-Peter C, Bidlingmaier M, et al. Low-dose ghrelin infusion--evidence against a hormonal role in food intake. Regulatory peptides. 2012;174(1-3):26-31. Epub 2011/11/29.

37. Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, et al. Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. European journal of endocrinology. 2008;158(4):491-8. Epub 2008/03/26.

38. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2004;89(6):2832-6. Epub 2004/06/08.

39. le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. J Clin Endocrinol Metab. 2005;90(2):1068-71. Epub 2004/11/04.

40. Martins C, Kulseng B, King NA, Holst JJ, Blundell JE. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. J Clin Endocrinol Metab. 2010;95(4):1609-16.

41. Burns SF, Broom DR, Miyashita M, Mundy C, Stensel DJ. A single session of treadmill running has no effect on plasma total ghrelin concentrations. Journal of sports sciences. 2007;25(6):635-42. Epub 2007/04/25.

42. Kraemer RR, Durand RJ, Acevedo EO, Johnson LG, Kraemer GR, Hebert EP, et al. Rigorous running increases growth hormone and insulin-like growth factor-I without altering ghrelin. Experimental biology and medicine (Maywood, NJ). 2004;229(3):240-6. Epub 2004/02/28.

43. Schmidt A, Maier C, Schaller G, Nowotny P, Bayerle-Eder M, Buranyi B, et al. Acute exercise has no effect on ghrelin plasma concentrations. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2004;36(3):174-7. Epub 2004/04/02.

44. Erdmann J, Tahbaz R, Lippl F, Wagenpfeil S, Schusdziarra V. Plasma ghrelin levels during exercise - effects of intensity and duration. Regulatory peptides. 2007;143(1-3):127-35. Epub 2007/06/16.

45. Ebrahimi M, Rahmani- Nia F, Damirchi A, Mirzaie B, Asghar Pur S. Effect of Short-term Exercise on Appetite, Energy Intake and Energy-regulating Hormones. Iranian Journal of Basic Medical Sciences. 2013;16(7):829-34.

46. Guelfi KJ, Donges CE, Duffield R. Beneficial effects of 12 weeks of aerobic compared with resistance exercise training on perceived appetite in previously sedentary overweight and obese men. Metabolism. 2013;62(2):235-243.

47. 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. New England Journal of Medicine. 2002;346(21):1623-30.

48. Friedenreich CM, Neilson HK, O'Reilly R, Duha A, Yasui Y, Morielli AR, et al. Effects of a High vs Moderate Volume of Aerobic Exercise on Adiposity Outcomes in Postmenopausal Women: A Randomized Clinical Trial. JAMA oncology. 2015;1(6):766-76. Epub 2015/07/17.

49. Slentz CA, Duscha BD, Johnson JL, et al. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: Strride—a randomized controlled study. Archives of Internal Medicine. 2004;164(1):31-9.

50. Donnelly JE, Hill JO, Jacobsen DJ, et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: The midwest exercise trial. Archives of Internal Medicine. 2003;163(11):1343-50.

51. Church TS, Martin CK, Thompson AM, Earnest CP, Mikus CR, Blair SN. Changes in Weight, Waist Circumference and Compensatory Responses with Different Doses of Exercise among Sedentary, Overweight Postmenopausal Women. PLOS ONE. 2009;4(2):e4515.

52. van Gemert WAM, Schuit AJ, van der Palen J, May AM, Iestra JA, Wittink H, et al. Effect of weight loss, with or without exercise, on body composition and sex hormones in postmenopausal women: the SHAPE-2 trial. Breast Cancer Research : BCR. 2015;17(1):120.

53. Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, et al. Mice lacking ghrelin receptors resist the development of diet-induced obesity. The Journal of clinical investigation. 2005;115(12):3564-72. Epub 2005/12/03.

54. Ebal E, Cavalie H, Michaux O, Lac G. Effect of a moderate exercise on the regulatory hormones of food intake in rats. Appetite. 2007;49(2):521-4. Epub 2007/04/28.