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An Examination of the Relationships Among Cardiorespiratory Fitness Status, Body Mass Index, Appetite and Ghrelin

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**AN EXAMINATION OF THE RELATIONSHIPS
AMONG CARDIORESPIRATORY FITNESS STATUS,
BODY MASS INDEX, APPETITE AND GHRELIN**

BY

JENNIFER ARTS

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
NUTRITION AND FOOD SCIENCES**

UNIVERSITY OF RHODE ISLAND

2009

MASTER OF SCIENCE THESIS

OF

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UNIVERSITY OF RHODE ISLAND

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ABSTRACT

Problem Statement: Obesity and its related metabolic disorders rank among the leading causes of illness and mortality worldwide. The prevalence of obesity has led researchers to focus on body weight regulation and the balance between energy intake and energy expenditure.

Background: Studies have suggested that ghrelin is involved in both short and long term energy balance. Previous research has examined the relationships among BMI, ghrelin and appetite but few studies have investigated the role that fitness status plays in relation to these.

Methods: Data from two studies that were previously conducted in the Energy Metabolism Laboratory were combined for a secondary data analysis. One hundred nine subjects (87 females, 22 males; 19.4 ± 2.1 years; $\text{BMI } 22.9 \pm 3.9 \text{ kg/m}^2$) were included in the analysis. Cardiorespiratory fitness (VO_2max) was estimated by the Queen's College Step test and self-reported physical activity was determined by the International Physical Activity Questionnaire. Ratings of appetite (by 100mm visual analog scales), plasma total ghrelin (by RIA) and insulin (by ELISA) were analyzed following a 10h fast and 30min after a standardized meal.

Results: Ghrelin was significantly correlated with BMI in both the fasting ($r=-0.290$, $p=0.005$) and fed ($r=-0.381$, $p=0.001$) states. Significant negative correlations were also found between VO_2max and both fasting ghrelin ($r=-0.305$, $p=0.003$) and fed ghrelin ($r=-0.336$, $p=0.005$). Fasting ghrelin was also significantly correlated with vigorous physical activity ($r=-0.242$, $p=0.019$), waist circumference ($r=-0.298$, $p=0.004$) and insulin ($r=-0.324$, $p=0.012$). Ghrelin and appetite were not significantly

related at single time point measures (t=0, 30 min). BMI was positively correlated with dietary restraint ($r=0.258$, $p=0.007$) and disinhibition ($r=0.276$, $p=0.004$), whereas $VO_2\text{max}$ was negatively correlated with dietary restraint ($r=-0.350$, $p<0.001$). ANOVA indicated that subjects with BMIs above the median had significantly lower fasting ghrelin (692.2 ± 237.9 pg/mL vs. 802.2 ± 266.0 pg/mL, $p=0.037$) and fed ghrelin (575.0 ± 164.4 pg/mL vs. 710.2 ± 226.4 pg/mL, $p=0.005$) and significantly greater disinhibition (6.5 ± 3.1 vs. 4.8 ± 3.1 , $p=0.005$) compared to those with BMIs below the median. Subjects with a higher fitness status reported significantly lower fasting satiety (31.4 ± 22.3 vs. 36.8 ± 22.2 , $p=0.022$), greater desire to eat (53.3 ± 17.5 vs. 45.5 ± 20.5 , $p=0.037$), and less dietary restraint (7.8 ± 5.2 vs. 11.3 ± 5.4 , $p=0.001$) compared to subjects with a lower fitness status.

Significance and Conclusions: These findings confirm previous relationships reported between BMI and ghrelin, subjective appetite and eating behaviors and corroborate lack of associations between single-point time measures of ghrelin and appetite. Additionally, results indicate that cardiorespiratory fitness may be related to circulating ghrelin levels, subjective appetite, and degree of dietary restraint. Further study is needed to determine the nature of these relationships.

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equally as important as department assignments. Cathy made my entire graduate experience a positive one.

PREFACE

This thesis is a secondary data analysis examining the relationships among cardiorespiratory fitness status, BMI, ghrelin and appetite. The thesis is presented in manuscript format. The manuscript was formatted for submission to the journal *Physiology and Behavior*.

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MANUSCRIPT

ABSTRACT

Studies have suggested that ghrelin is involved in both short and long term energy balance. Previous research has examined the relationships among BMI, ghrelin and appetite but few studies have investigated the role that fitness status plays in relation to these variables. Data from two studies that were previously conducted in our laboratory were combined for a secondary data analysis. One hundred nine subjects (87 females, 22 males; 19.4 ± 2.1 years; BMI 22.9 ± 3.9 kg/m²) were included in the analysis. Cardiorespiratory fitness was estimated by the 3 minute Queen's College Step test and the 51-item Three Factor Eating Questionnaire was used to assess cognitive restraint, disinhibition and perceived hunger. Ratings of appetite (by 100 mm visual analogue scale), plasma total ghrelin (by RIA) and insulin (by ELISA) were analyzed following a 10 h fast and 30 min after a standardized meal. Ghrelin was significantly correlated with BMI in both the fasting ($r=-0.290$, $p=0.005$) and fed ($r=-0.381$, $p=0.001$) states. Significant negative correlations were also found between estimated cardiorespiratory fitness (VO₂max) and both fasting ghrelin ($r=-0.305$, $p=0.003$) and fed ghrelin ($r=-0.336$, $p=0.005$). Fasting ghrelin was also significantly correlated with vigorous physical activity ($r=-0.242$, $p=0.019$; by IPAQ), waist circumference ($r=-0.298$, $p=0.004$) and insulin ($r=-0.324$, $p=0.012$). Ghrelin and appetite ratings were not significantly related at single time point measures ($t=0,30$ min). BMI was positively correlated with dietary restraint ($r=0.258$, $p=0.007$) and disinhibition ($r=0.276$, $p=0.004$), whereas VO₂max was negatively correlated with dietary restraint ($r=-0.350$, $p<0.001$). ANOVA indicated that subjects with BMIs

above the median had significantly lower fasting ghrelin (692.2 ± 237.9 pg/mL vs. 802.2 ± 266.0 pg/mL, $p=0.037$) and fed ghrelin (575.0 ± 164.4 pg/mL vs. 710.2 ± 226.4 pg/mL, $p=0.005$) and significantly greater disinhibition (6.5 ± 3.1 vs. 4.8 ± 3.1 , $p=0.005$) compared to those with BMIs below the median. Subjects with a higher cardiorespiratory fitness status reported significantly lower fasting satiety (31.4 ± 22.3 vs. 36.8 ± 22.2 , $p=0.022$), greater desire to eat (53.3 ± 17.5 vs. 45.5 ± 20.5 , $p=0.037$), and less dietary restraint (7.8 ± 5.2 vs. 11.3 ± 5.4 , $p=0.001$) compared to subjects with a lower fitness status. These findings confirm previous relationships reported between BMI and ghrelin, subjective appetite and eating behaviors and corroborate lack of associations between single-point time measures of ghrelin and appetite. Additionally, results indicate that cardiorespiratory fitness may be related to circulating ghrelin levels, subjective appetite, and degree of dietary restraint. Further study is needed to determine the nature of these relationships.

INTRODUCTION

Obesity and its related metabolic disorders rank among the leading causes of illness and mortality worldwide [1]. The prevalence of obesity has led researchers to focus on body weight regulation and the balance between energy intake and energy expenditure. Ghrelin is a recently discovered hormone that appears to be associated with both short and long term body weight regulation [2]. Ghrelin plays an important role as a short term regulator of appetite through its involvement in meal initiation. Plasma ghrelin levels rise before meals and decrease after meals [3]. Studies on rodents indicate that ghrelin infusion not only promotes weight gain by increasing food intake but also by decreasing energy expenditure and fat catabolism [4-6]. Intravenous infusion of ghrelin in humans increased food intake in both lean and obese subjects [7]. In addition, higher ghrelin levels have been linked to lower resting metabolic rate and thermic effect of food [8, 9]. Collectively, these findings support a role for ghrelin in both energy intake and in energy expenditure.

Ghrelin levels fluctuate with body weight. Research has demonstrated that circulating concentrations are low in obese individuals and increase with weight loss [10, 11]. Due to the role that exercise plays in weight loss, recent studies have explored the effects that exercise has on ghrelin [11-15]. Exercise is an established method to increase energy expenditure. Because exercise training improves fitness status, promotes weight loss and improves overall health, there has been recent research devoted to examining the effects of exercise on ghrelin levels. A chronic exercise-induced energy deficit may be associated with higher ghrelin levels. This was demonstrated in long term exercise interventions [11, 16]. These increases, however,

often occur along with weight loss so it is difficult to determine if the increases in ghrelin are due to the weight loss or to physical activity itself. More research is necessary to determine the mechanism responsible for this increase.

Understanding body weight regulating hormones is also essential in determining the role that fitness plays in regulating appetite. Hunger suppression is observed immediately after exercise [17-19] but compensatory effects on food intake occur in the long run to compensate for an average of 30% of energy expended in physical activity [19]. Other research has also explored the relationship between dietary restraint and exercise. It was hypothesized that high dietary restraint would be associated with a tendency for disinhibition and increased post-exercise energy intake [20]. This was not observed in overweight males [20] or in female dieters [21]. This may be attributed to the fact that increases in energy expenditure from physical activity may not be immediately compensated for by increases in energy intake [22]. If fitness status modifies these relationships has yet to be determined.

Few studies have directly examined fitness status and its relation to ghrelin. The question of whether fit individuals have higher levels of ghrelin has yet to be fully investigated. The primary purpose of this study is to examine relationships among fitness, BMI, and ghrelin and to determine their independent effects on appetite. A better understanding of these relationships will provide insight into the mechanisms that govern body weight regulation and the role that exercise training plays in this process. Determining if ghrelin levels are a function of fitness status may also clarify the relationships between fitness and appetite. This study will also assist researchers in planning human subject studies based on subject characteristics.

METHODS

Participants

Subjects were normal weight, healthy male and female volunteers recruited from the university community using flyers, class announcements and newspaper advertisements. All participants provided voluntary, written informed consent. The University of Rhode Island Institutional Review Board approved the experimental protocol and procedures.

Study design

Data sets from two studies that were previously conducted in our laboratory were combined for a secondary data analysis including one hundred nine subjects (n=87 females, n=22 males). The Ghrelin and Appetite Response to Feeding (GARF) study consisted of seventy-two females and twenty-two males. Exclusion criteria included: an inability to donate blood due to anxiety or a bleeding disorder, an allergy to any component of the test meal, pregnancy or lactation, and age younger than 18 years. Participants reported to the laboratory after a 12-hour overnight fast. Visual analogue scales, which were used to assess hunger, satiety and desire to eat were completed before and 30 minutes after the consumption of a breakfast shake (Boost, Novartis Medical Nutrition, Minneapolis, MN). The breakfast shake contained 68.3% carbohydrate, 16.7% protein and 15% fat and provided 25% of each participant's estimated resting energy expenditure as calculated by the WHO equation (males= $(15.3 \times \text{wt}) + 679$), (females= $(14.7 \times \text{wt}) + 746$) (WHO, 1985). The average kilocalorie content of the shake was 448.1 kcal for males and 347.9 kcal for females. Fasting and

30 minute postprandial blood samples were obtained from an antecubital vein for analysis of ghrelin, glucose, and insulin.

The GRAIN Study consisted of seventeen females ages 18-26 years. Exclusion criteria included: any disorder or medication known to influence metabolism or appetite, clinically-diagnosed insulin resistance, diabetes mellitus, serious illnesses, history of or current clinically-diagnosed eating disorder, weight-loss of more than five pounds in the last three months, dependence on caffeine or alcohol, allergies to any of the test foods, pregnancy or lactation.

Participants reported to the laboratory after a 10-hour overnight fast. Visual analogue scales were administered and blood draws were performed in both the fasting state and at 15, 30, 45, 60, 90, 120, 150 and 180 minutes postprandially. Only the 30 minute time point was used for the purpose of this secondary data analysis. After the fasting measures, participants were served a test meal consisting of 15 g of a generic corn chex cereal, 15 g of puffed rice cereal, and 54 g (2 slices) of white (wheat) toast. Strawberry jam was weighed and spread on the toast and milk (1% fat for the whole grain condition and 2% for the refined grain condition) was measured and poured over the cereal. The test meal consisted of 71% carbohydrate, 15% fat, 14% protein and provided 427.2 kcal.

Fitness and physical activity

In both protocols, cardiorespiratory fitness was estimated using the Queen's College Step Test [23]. Subjects were asked to step up and down on a 16.25 inch box to the beat of a metronome for three minutes. Post exercise heart rate was measured by palpation of the radial artery for 15 seconds immediately after and this number was

multiplied by 4 to obtain heart rate in beats/minute and then put into the gender-specific equations for estimated VO_2max [23]. A new equation that was developed at the University of Rhode Island was used to more accurately estimate VO_2max in male participants (Deborah Riebe, personal communication). To account for gender differences in VO_2max , the sample was grouped into fitness categories according to ACSM gender-specific cutoffs.

Self-reported physical activity was assessed using the short self-administered format of the International Physical Activity Questionnaire (IPAQ) [24]. Participants were asked to report their walking, moderate, and vigorous physical activity over the past seven days.

Blood analytes: ghrelin, glucose, insulin

Blood draws for the GARF study were performed by venipuncture, following a 12-hour overnight fast and 30 minutes after breakfast. Blood samples were drawn into two 2 mL EDTA tubes (Vacurette, Greiner Bio-One, Monroe, NC). The tube for analysis of ghrelin was treated with 50 μL Aprotinin/mL (Phoenix Pharmaceuticals, Belmont, CA, Catalog # RK-APRO; Lot # 422979; Dose 30.0 Trypsin Inhibitor Unit/5mL). The other tube for analysis of glucose and insulin was left untreated. Both tubes were then spun at 3200 rpm for 10 minutes on a bench top centrifuge (Unico Power Spin, Dayton, NJ). The plasma supernatant was aliquoted and kept at -20°C until frozen and then was transferred to a -80°C freezer for storage until batch analysis. Plasma glucose was analyzed using a Cobas Mira Plus blood analyzer (Roche Diagnostics, Branchburg, NJ). Total ghrelin was analyzed using a radioimmunoassay kit (GHRT-89HK; Linco Research, St. Charles, MO). Plasma

insulin was measured by enzyme-linked immunosorbent assay (ELISA) (Merckodia, Uppsala, Sweden).

Blood draws for the GRAIN study were performed using a flexible in-dwelling catheter placed in the antecubital vein in the non-dominant arm through a three-way stop-cock kept patent with sterile saline. Blood draws occurred after a 10-hour overnight fast and following the test meal at 15, 30, 45, 60, 90, 120, 150, 180 minutes postprandial. Blood glucose levels were measured immediately using whole venous blood samples (Hemocue Auto-Analyzer, Mission Viejo, CA). Blood samples for analysis of ghrelin and insulin were centrifuged immediately at 3800 rpm for 8 minutes. After centrifuging, 600 μ L of plasma supernatant was transferred to a 1.5 mL microcentrifuge tube pre-treated with 6 μ L of 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride. Thirty μ L of 1-Normal hydrochloric acid was then added. A separate untreated aliquot was used for insulin analysis. Tubes were frozen at -80°C . Total ghrelin was analyzed using a radioimmunoassay kit (GHRT-89HK; Linco Research, St. Charles, MO). Plasma insulin was measured by ELISA (Merckodia, Uppsala, Sweden).

Body mass index

Body mass index was calculated from height and weight measurements. Height was measured to the nearest millimeter without shoes using a wall-mounted stadiometer (Seca Acu-Height, Hamburg, Germany). Weight was measured to the nearest quarter of a pound using a calibrated physician's scale (Detecto, Webb City, MO) in the GARF study and was measured to the nearest kilogram on a calibrated

digital scale that was part of the BODPOD system in the GRAIN study (BodPod, model 2000A, Life Measurement Incorporated, Concord, CA).

Waist circumference

Waist circumference was measured in centimeters at the level of the umbilicus after normal expiration using a physician's tape measure.

Appetite

Volunteers were asked to rate their subjective appetite (hunger, satiety, desire to eat) ranging from "not at all" to "extremely" on a 10 cm validated visual analogue scale (VAS) [25]. Appetite assessments were administered in both fasting and fed states.

The 51-item Three Factor Eating Questionnaire (TFEQ) was also administered to measure cognitive dietary restraint, tendency for disinhibition, and perceived general hunger levels [26].

Body composition

Body composition was assessed in the Grain Study after a four hour fast using air displacement plethysmography in a self-contained system using a computer-integrated dual-chambered air plethysmograph equipped with a digital scale (BodPod, model 2000A, Life Measurements Incorporated, Concord, CA). This instrument was calibrated before each test according to manufacturer specifications. Subjects were asked to void their bladders and remove any jewelry before testing. Subjects wore a lycra swim cap and swimsuit during testing. Corrections were made for thoracic lung volume. Repeat measures were taken and percent body fat and lean body mass were

calculated from body volume using the Siri equation [27]. This technique of measuring body composition has been validated in a wide-range of populations [28].

Statistical analyses

All analyses were performed using SPSS version 16.0 for Windows. Pearson correlations were used to describe relationships among continuous variables. BMI and VO₂max were also analyzed categorically. A median split was used for BMI. VO₂max groups were created according to the American College of Sports Medicine's (ACSM) gender-specific cutoffs ("lower fitness status" males: VO₂max 26.6-45.50 ml/kg/min females: VO₂max 22.6-38.80 ml/kg/min, "higher fitness status" males: VO₂max 45.51-61.2 ml/kg/min, females: VO₂max 38.81-55.0 ml/kg/min). ANOVA was performed to examine between group differences in fasting and fed ghrelin, in TFEQ restraint, hunger, and disinhibition, and in fasting satiety, desire to eat and hunger. Waist circumference, blood glucose, and insulin were entered as covariates in ANCOVA to examine their influence on the relationship between BMI and fasting and fed ghrelin. Partial correlations controlling for cardiorespiratory fitness, BMI, waist circumference, blood glucose and insulin were performed to determine their impact on the relationships between ghrelin and appetite in the fasting and fed states. Stepwise multiple regression analysis was then used to determine how well waist circumference, BMI, and insulin predicted fasting and fed ghrelin.

RESULTS

Subject Characteristics

One hundred nine subjects were included in the analysis (87 females, 22 males). The mean age was 19.4 ± 2.1 years. The mean BMI was 22.9 ± 3.9 kg/m² and the median BMI was 22.0 kg/m². Estimated mean VO₂max was 40.3 ± 8.5 ml/kg/min. The mean VO₂max of the lower fit group was 36.8 ± 2.3 ml/kg/min and the mean VO₂max of the higher fit group was 44.8 ± 5.2 ml/kg/min. Mean values for fasting and fed ghrelin were 745 ± 256 pg/mL and 634 ± 204 pg/mL, respectively. Subject characteristics are presented in Table 1.

Relationships among BMI, cardiorespiratory fitness, physical activity, and ghrelin

As shown in Figures 1 and 2, ghrelin was significantly correlated with BMI in both the fasting ($r=-0.290$, $p=0.005$) and fed ($r=-0.381$, $p=0.001$) states. Significant negative correlations were also found between estimated cardiorespiratory fitness (VO₂max) and both fasting ghrelin ($r=-0.305$, $p=0.003$) and fed ghrelin ($r=-0.336$, $p=0.005$) as seen in Figure 3. Fasting ghrelin was also significantly correlated with reported vigorous physical activity ($r=-0.242$, $p=0.019$). Estimated cardiorespiratory fitness was also correlated with moderate ($r=0.249$, $p=0.009$), vigorous ($r=0.443$, $p<0.001$), and total ($r=0.369$, $p<0.001$) reported physical activity. ANOVA indicated that subjects with BMIs above the median had significantly lower fasting ghrelin (692.2 ± 237.9 pg/mL vs. 802.2 ± 266.0 pg/mL, $p=0.037$) and fed ghrelin (575.0 ± 164.4 pg/mL vs. 710.2 ± 226.4 pg/mL, $p=0.005$) compared to those with BMIs below the median. Refer to Table 2. There were no significant differences in either fasting

ghrelin ($p=0.099$) or fed ghrelin ($p=0.107$) between higher and lower fitness categories.

Relationships among BMI, cardiorespiratory fitness, physical activity, and appetite

BMI was positively correlated with dietary restraint ($r=0.258$, $p=0.007$) and disinhibition ($r=0.276$, $p=0.004$), whereas $VO_2\max$ was negatively correlated with dietary restraint ($r=-0.350$, $p<0.001$). $VO_2\max$ was positively correlated with fasting desire to eat ($r=0.327$, $p=0.001$). Physical activity was not significantly associated with subjective hunger, satiety, desire to eat or with dietary restraint, disinhibition or hunger.

Fasting desire to eat was associated with fasting hunger ($r=0.801$, $p<0.001$), with fasting satiety ($r=-0.394$, $p<0.001$), and with dietary restraint ($r=-0.195$, $p=0.042$). Fasting satiety was negatively correlated with fasting hunger ($r=-0.508$, $p<0.001$) and positively correlated with dietary restraint ($r=0.274$, $p=0.004$). In addition, dietary disinhibition was positively correlated with both dietary restraint ($r=0.265$, $p=0.005$) and with TFEQ hunger ($r=0.621$, $p<0.001$)

Subjects with BMIs above the median had significantly greater disinhibition (6.5 ± 3.1 vs. 4.8 ± 3.1 , $p=0.005$) compared to those with BMIs below the median. As shown in Table 3, subjects with a higher fitness status reported significantly lower fasting satiety (31.4 ± 22.3 vs. 36.8 ± 22.2 , $p=0.022$), greater desire to eat (53.3 ± 17.5 vs. 45.5 ± 20.5 , $p=0.037$), and less dietary restraint (7.8 ± 5.2 vs. 11.3 ± 5.4 , $p=0.001$) compared to subjects with a lower fitness status. Ghrelin and appetite were not significantly related at single time point measures ($t=0$, 30 min) and this relationship

was not modified by cardiorespiratory fitness, BMI, waist circumference, blood glucose or insulin.

Relationships among BMI, waist circumference, % body fat, insulin and ghrelin

BMI was highly correlated with waist circumference ($r=0.836$, $p<0.001$). Fasting ghrelin was negatively correlated with BMI ($r=-.290$, $p=0.005$), waist circumference ($r=-0.298$, $p=0.004$) and insulin ($r=-0.324$, $p=0.012$). The relationship between fasting ghrelin and BMI was no longer significant ($p=0.455$) after controlling for waist circumference. Controlling for blood glucose and insulin did not modify the relationship between BMI and fasting ghrelin. In the fed state, controlling for waist circumference, blood glucose, and insulin did not have an effect on the relationship between BMI and ghrelin. In addition, ANCOVA revealed that differences in fasting ghrelin between BMI groups were no longer significant after controlling for waist circumference, insulin, and blood glucose. Significant differences remained, however, in the fed state even after controlling for these variables. Percent body fat ($n=17$) was not significantly related to fasting ghrelin ($p=0.378$) or fed ghrelin ($p=0.279$). Also, % body fat did not modify the relationship between ghrelin and appetite in the fasting or fed state.

Predictors of fasting ghrelin

Three models were developed using the variables that were significantly correlated with ghrelin to predict ghrelin levels. The most significant model ($p<0.001$)

was using BMI and VO₂max to predict fasting ghrelin. Models are presented in Table

4.

DISCUSSION

The results indicate that there is a significant inverse relationship between both BMI and ghrelin and between VO_2 max and ghrelin in the fed and fasting state. The appetite findings suggest that there are also links among eating behaviors, appetite and both BMI and VO_2 max. Subjects with BMIs above the median had significantly higher disinhibition scores compared to those with BMIs below the median. More fit subjects reported significantly lower fasting satiety, greater desire to eat, and less dietary restraint compared to less fit subjects.

The finding that BMI is negatively associated with ghrelin is in agreement with previous studies [29, 30]. Similarly, negative relationships between ghrelin and waist circumference [29] and insulin [30] have previously been reported. The relationship between fasting ghrelin and BMI was no longer significant after statistical control for waist circumference. Waist circumference was slightly more correlated with ghrelin than BMI was. In addition, waist circumference and BMI were highly correlated ($r=0.836$, $p<0.001$). This is similarly reported in the literature ($r>0.8$ in most studies) [31]. This is useful for future studies and suggests that BMI and waist circumference may be similar indexes of body composition [31].

The significant negative association that was found between VO_2 max and ghrelin was inconsistent with our hypotheses. It was counterintuitive that the associations between ghrelin and BMI and ghrelin and VO_2 max were both negative. It was hypothesized that the more fit individuals would have higher ghrelin levels. Previous research has indicated that ghrelin increases in response to a negative energy balance [11, 32]. The literature on the effects of short term exercise on ghrelin is

inconsistent. Most studies failed to find an effect of exercise on ghrelin levels [12, 33-36] but two acute exercise studies have reported increases in ghrelin immediately following exercise [13, 14]. The conflicting results in differences in protocols make it difficult to reach a consensus.

Research on chronic exercise, however, suggests that ghrelin levels rise from long term exercise interventions [11, 16]. These findings are complicated by the fact that weight loss also occurred along with the increases in ghrelin, making it difficult to determine if the increases were due to weight loss or to physical activity itself. Weight loss is known to result in increased ghrelin levels [3, 32]. These increases are commensurate with the amount of weight lost [11]. There is some evidence that there may not be an independent effect of exercise on ghrelin apart from weight loss. A study that included a weight stable group found that three months of exercise training had no effect on ghrelin levels in the absence of weight loss [10].

The reason for the negative correlation between ghrelin and $VO_2\text{max}$ is unknown. Other studies that have analyzed $VO_2\text{max}$ and ghrelin are inconclusive. An aerobic exercise intervention involving 173 sedentary, overweight postmenopausal women that compared changes in ghrelin levels to changes in fitness levels (changes in $VO_2\text{max}$) from baseline to 12 months found a non-significant negative association between ghrelin and fitness level [11]. Another study conducted on 65 normal weight females found a non-significant positive relationship between fasting ghrelin and $VO_2\text{max}$ [8]. The authors also found significant negative associations between ghrelin and RMR and TEF independent of fat mass and fat free mass. A more recent study found that ghrelin was negatively associated with RMR in non-obese women

independent of body weight [9]. The authors suggested that ghrelin may be regulated by voluntary long-term behavior modification that increases RMR such as aerobic and resistance training [37]. This hypothesis would be consistent with the negative association found between fitness status and ghrelin in our study.

Significant differences in appetite and eating behavior were observed between BMI and fitness categories. Subjects with a higher BMI had greater disinhibition compared to subjects with a lower BMI. This finding was consistent with the literature [38]. Disinhibition is positively correlated with both BMI and obesity. It is also predictive of less successful weight loss attempts, weight regain, and low physical activity [38]. BMI has also been previously associated with dietary restraint [39, 40]. A significant positive correlation was found between BMI and dietary restraint in our study but there were no significant differences in dietary restraint between BMI groupings.

Subjects with a higher fitness status had significantly lower fasting satiety, greater desire to eat and less dietary restraint. These findings are interesting from a behavioral standpoint. There is a widespread belief that the energy expended from exercise can offset the consumption of more energy dense foods [41]. More fit individuals may be more likely to adopt less stringent diets due to the perception that the additional calories consumed will be expended during exercise. This perception could potentially lead to weight gain in individuals engaging in minimal exercise who feel that they do not have to be as conscious of their energy intake. Alternatively, more fit individuals might not need to restrain their energy intake to regulate body weight.

Further research should be conducted to determine if lower dietary restraint corresponds to an increase in energy intake in fit individuals.

No significant relationship was found between percent body fat and ghrelin. Previous research reported that ghrelin was negatively correlated with both percent body fat and fat mass [42]. However, in our analysis, since percent body fat data were only available for 17 of the 109 subjects it was difficult to draw conclusions based on this small sub-sample. It would be interesting to examine how percent body fat modifies relationships between ghrelin and appetite on a larger scale.

This study failed to find a significant difference in either fasting or postprandial ghrelin between high and low fitness categories. Also, there was no association found between ghrelin and subjective ratings of appetite. Previous studies have also failed to find an association [43, 44]. This may be due to the difficulty in finding relationships between plasma ghrelin concentrations and single time point measures of appetite. This also holds true for single time point measures of other biomarkers of satiety [45].

The conclusions are limited by several factors. First, the gender distribution was unbalanced. Males have an inherently greater VO_2max . To account for the gender differences in VO_2max , the sample was grouped into fitness categories according to ACSM gender-specific cutoffs. Most other studies only include one gender. The estimation instead of the direct measurement of VO_2max was another limitation. Also, there were missing data from the blood draws resulting in 94 subjects with fasting ghrelin values, 69 subjects with postprandial ghrelin values, and 60 subjects with fasting and postprandial insulin values. Another limitation may be that there were

relatively few subjects with a high $VO_2\text{max}$ and a high BMI. In addition, because this was a secondary data analysis there were differences in experimental protocols between the two studies. For example, the test meals that were provided in each study following fasting measures, were standardized but differed in composition. In the GARF study, participants consumed a breakfast shake that contained 68.3% carbohydrate, 16.7% protein, 15% fat and provided 25% of each participant's estimated resting energy expenditure as calculated by the WHO equation (males= $(15.3 \times \text{wt}) + 679$, females= $(14.7 \times \text{wt}) + 746$) (WHO, 1985). The average kilocalorie content of the shake was 448.1 kcal for males and 347.9 kcal for females. In the GRAIN study, the refined grain test meal that was used in this analysis consisted of corn chex and puffed rice cereal with milk and white toast with strawberry jam. This test meal provided 71% carbohydrate, 15% fat, 14% protein and provided 427.2 kcal. Although the total kilocalories and macronutrient composition of the test meals were similar, the perception of satiety may have been altered due to the fact that one test meal was a shake and one was a solid meal.

The strength of the relationships that were found between BMI and ghrelin and between $VO_2\text{max}$ and ghrelin were evidenced by multiple regression analysis. The model including BMI and $VO_2\text{max}$ as the only predictors of fasting ghrelin proved to be the most significant model. This supports the correlation results.

This study contributes to the scientific literature because it is the largest single analysis to our knowledge that examined relationships of both BMI and $VO_2\text{max}$ to both fasting and fed ghrelin. Although many of the variables that were included in this analysis were previously studied in relation to ghrelin, the relationships among BMI,

VO_2 max, ghrelin and appetite have not been directly compared. In addition, the inclusion of both genders and the use of both fasting and postprandial ghrelin data were unique features of this study. The nature of the secondary data analysis also allowed for a larger sample size (n=109) compared to other studies in this field.

These findings confirm previous relationships reported between BMI and ghrelin, subjective appetite and eating behaviors and corroborate lack of associations between single-point time measures of ghrelin and appetite. Additionally, results indicate that cardiorespiratory fitness is related to circulating ghrelin levels, subjective appetite, and degree of dietary restraint. Further study is needed to determine the nature of these relationships.

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Table 1: Subject characteristics

* VO₂max was estimated by the Queen's College Step Test

**Ghrelin was measured after a 10 hour overnight fast and 30 minutes after a standardized meal

Subject Characteristics	n	Mean ± SD
Age (years)	109	19.4 ± 2.1
BMI (kg/m ²)	109	22.9 ± 3.9
Estimated VO ₂ max (ml/kg/min)*	108	40.3 ± 5.6
Waist circumference (cm)	109	79.6 ± 10.0
Fasting ghrelin (pcg/mL)**	94	745 ± 256
Fed ghrelin (pcg/mL)**	69	634 ± 204

Table 2: Mean ghrelin levels by BMI category

	Below median		Above median		P value
	n	mean	n	mean	
Fasting ghrelin, pg/mL	45	802 ± 266	49	692 ± 238	0.037
Fed ghrelin, pg/mL	30	710 ± 226	39	575 ± 164	0.005

Table 3: Mean appetite scores by fitness category

*VAS range: 0-100

**TFEQ ranges: restraint 0-21
disinhibition 0-13
hunger 0-13

	Cardiorespiratory Fitness Category		P value
	Lower Fit (n=60)	Higher Fit (n=48)	
VAS fasting hunger*	46.58 ± 25.98	51.93 ± 24.92	0.281
VAS fasting satiety*	41.10 ± 21.22	31.35 ± 22.34	0.022
VAS fasting desire to eat*	45.48 ± 20.48	53.33 ± 17.47	0.037
TFEQ Restraint**	11.28 ± 5.40	7.81 ± 5.16	0.001
TFEQ Disinhibition**	5.85 ± 3.19	5.46 ± 3.27	0.532
TFEQ Hunger**	5.52 ± 2.90	5.77 ± 3.01	0.657

Table 4: Fasting ghrelin prediction equations

	Predictor (s)	B	β	t	p	R	R ²
Equation 1	Constant	1302.505		5.148	0.000		
	WC (cm)	-5.954	-0.231	-1.836	0.072		
	Insulin (mU/L)	-9.202	-0.265	-2.108	0.039	0.393	0.155
Equation 2	Constant	1211.412		6.555	0.000		
	BMI (kg/m ²)	-16.366	-0.250	-2.045	0.045		
	Insulin (mU/L)	-10.015	-0.289	-2.363	0.022	0.407	0.166
Equation 3	Constant	1829.320		7.725	0.000		
	BMI (kg/m ²)	-20.630	-0.315	-3.313	0.001		
	VO ₂ max (ml/kg/min)	-15.148	-0.328	-3.455	0.001	0.438	0.191

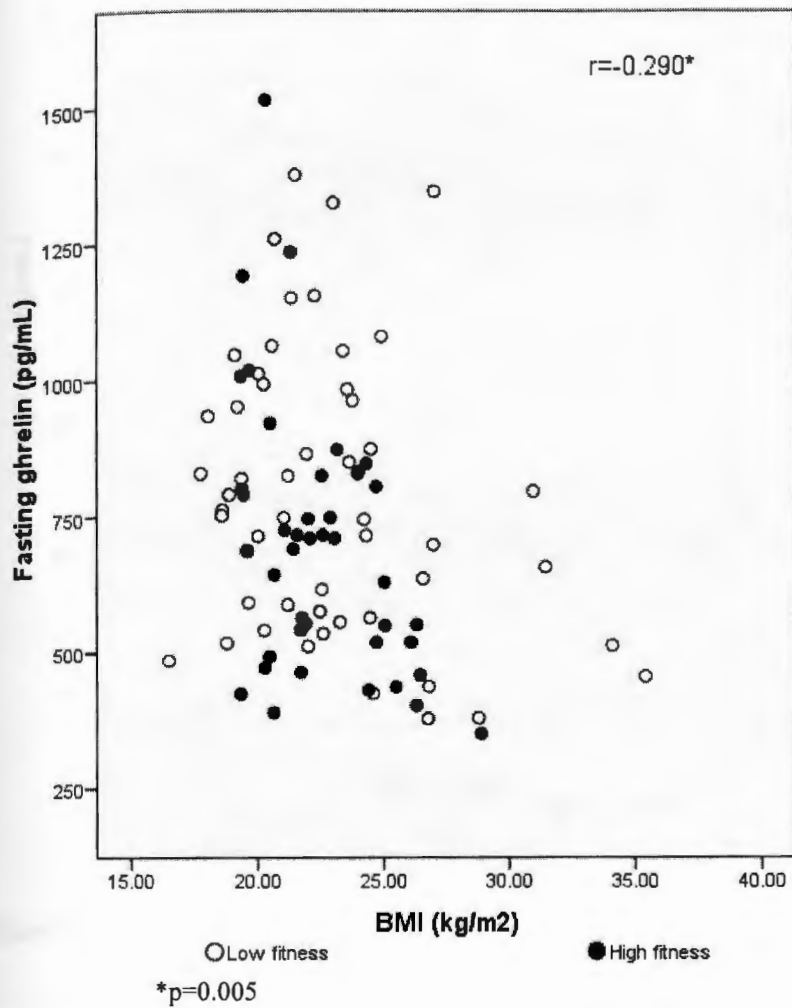


Figure 1: Relationship between fasting ghrelin and BMI

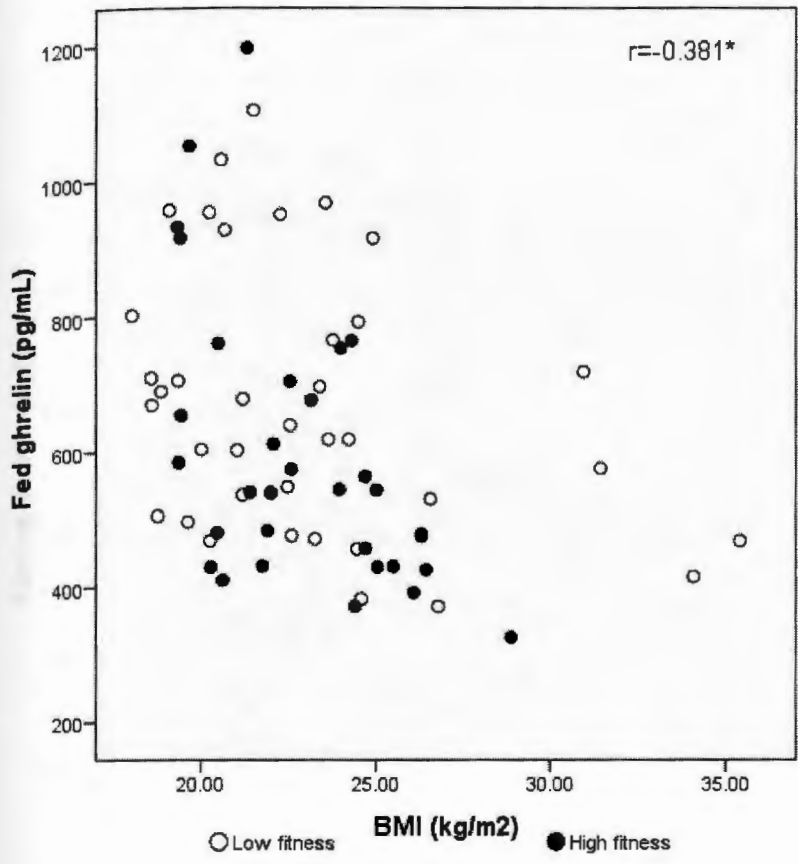
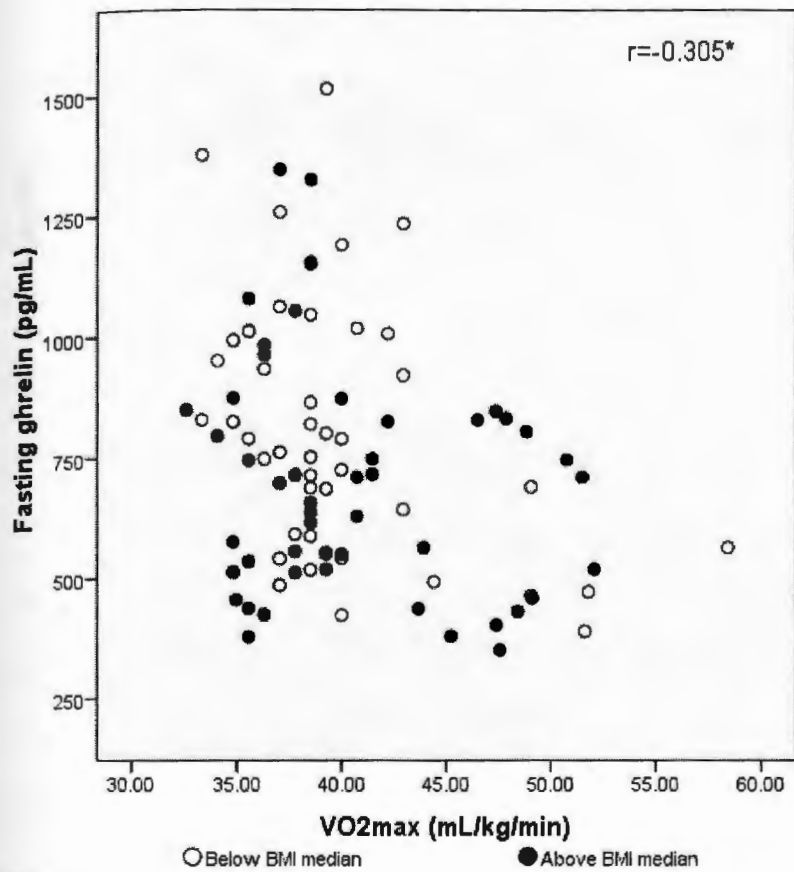


Figure 2: Relationship between fed ghrelin and BMI



*p=0.003

Figure 3: Relationship between fasting ghrelin and estimated cardiorespiratory fitness

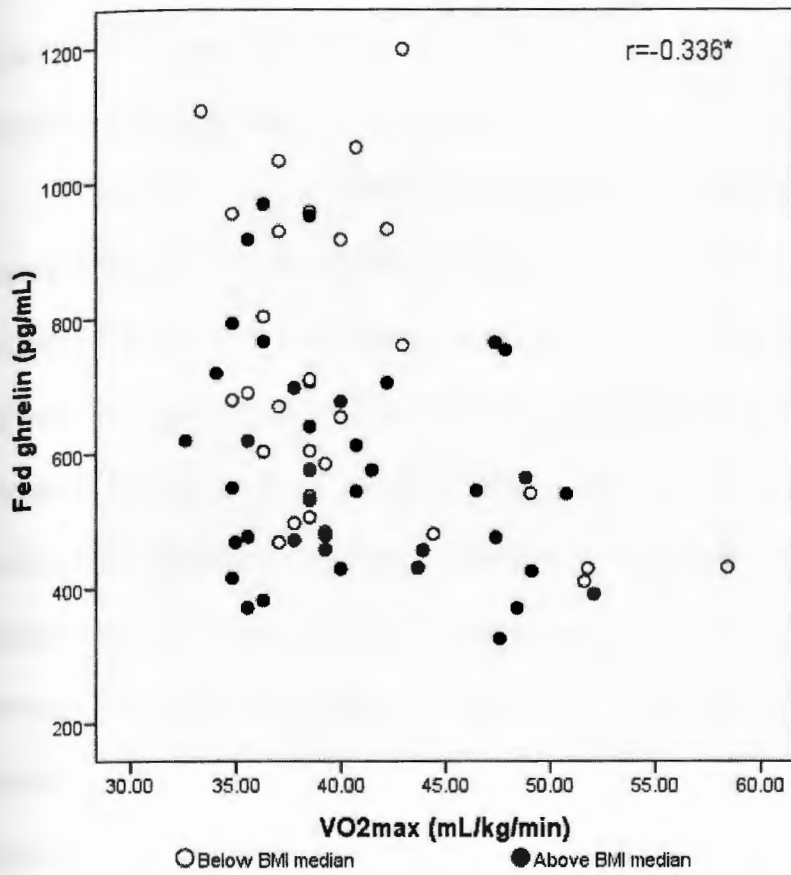


Figure 4: Relationship between fed ghrelin and estimated cardiorespiratory fitness

APPENDIX A

Literature Review

Ghrelin and Energy Balance

Ghrelin is a recently discovered hormone that regulates energy balance [1, 2]. Since its discovery in 1999, it has piqued much interest in the field of energy metabolism. Studies have indicated that ghrelin plays a pivotal role in both short term and long term energy balance [3]. Ghrelin is a hyperphagic hormone secreted by the stomach that participates in meal initiation [3]. Research also suggests that ghrelin is involved in long term energy balance. Ghrelin is associated with energy storage. Studies have found that ghrelin levels are elevated in individuals with anorexia nervosa [4] and levels are decreased in obese individuals [5]. This indicates that ghrelin is downregulated in response to excess energy stores. Ghrelin levels return to normal when a healthy body weight is achieved [6]. Hansen's research that measured fasting ghrelin in response to weight loss in obese subjects not only found that fasting ghrelin concentrations increased with weight loss but also found that this increase was positively correlated with the extent of weight loss [5]. In a six month diet program for weight loss, Cummings et al. found a 24 percent increase in the area under the curve for the 24-hour ghrelin profile with a diet induced weight loss of 17 percent [7]. This is consistent with the hypothesis that ghrelin has a role in the long term regulation of body weight [7]. A negative energy balance or weight loss threshold for increasing ghrelin, however, has yet to be determined [8].

Ghrelin and Appetite

Ghrelin stimulates appetite by acting on the arcuate nucleus in the hypothalamus. This region is responsible for controlling food intake [9].

Ghrelin plays an important role as a short term regulator of appetite through its involvement in meal initiation. Studies on rodents indicate that ghrelin infusion promotes weight gain by increasing food intake and decreasing energy expenditure and fat catabolism [10-12]. Intravenous infusion of ghrelin in humans increased food intake in both lean and obese subjects [13]. Endogenous ghrelin levels rise before meals to sufficient levels to stimulate appetite [3]. Postprandial ghrelin levels are quickly suppressed by nutrient ingestion [14]. This is evidenced by twenty-four hour plasma profiles that show these preprandial increases in ghrelin and postprandial decreases in ghrelin with every meal [14, 15].

A study that analyzed the short term effects of energy restriction on total fasting and postprandial ghrelin reported no significant changes in ghrelin after the four day energy restriction [16]. There were no associations found between total ghrelin and appetite scores. This suggests that ghrelin does not have an immediate effect on appetite in a typical hypocaloric dietary regimen.

Ghrelin Biochemistry

Ghrelin is synthesized predominantly by the stomach [17]. The fundus synthesizes approximately ten times more ghrelin per gram of tissue than other production sites including the duodenum and other parts of the small intestine [18]. Gastrectomy and small bowel resections have indicated that approximately 2/3 of

circulating ghrelin is secreted by the stomach with the rest being secreted by the small intestine [19]. Ghrelin is a peptide containing 28 amino acids. It undergoes post-translational modification that involves the acylation of the serine-3 residue. The serine residue is covalently attached to a medium-chain fatty acid by an ester bond. This acylation is necessary for ghrelin to bind and activate its receptor, the growth hormone secretagogue receptor (GHS-R) [9]. As a result, most of ghrelin's physiological roles are associated with its acylated form, commonly referred to as active ghrelin. Studies in mice lacking the GHS-R gene have suggested that this receptor is responsible for the orexigenic effects of ghrelin [20]. Ghrelin also exists, however, in another isoform, des-acyl ghrelin, which is suggested to play important roles in glucose and insulin metabolism [21-23] and in adipogenesis [24]. Research is being conducted to identify alternate ghrelin receptors that interact with the des-acylated form [2].

Ghrelin and Exercise

Research has been devoted to examining the effects of exercise on ghrelin due to ghrelin's role in chronic energy balance. Since exercise and ghrelin both affect energy balance, studies have been performed to determine if a relationship exists between the two. Ghrelin levels are increased in states of negative energy balance [5]. This increase may be part of an adaptive response to counteract a negative energy balance [25]. As a result, it has been hypothesized that the energy expenditure resulting from exercise may have an effect on ghrelin. Both short term studies [26-28] and long term studies [6, 25] have been performed to examine the effects of exercise

on ghrelin. Most of the studies examining acute exercise on ghrelin levels have failed to find an effect on ghrelin levels during or shortly after exercise. One study involving treadmill exercise at 50%, 70%, and 90% of VO_{2max} found no changes in ghrelin at any of the three intensities [26]. No changes in ghrelin were observed in a similar study involving a one hour treadmill bout at 73% of VO_{2max} compared to the control condition [29]. Other studies produced conflicting results. In one study, ghrelin was suppressed during both aerobic and resistance exercise conditions [30]. In contrast, another study reported a significant increase in ghrelin immediately after maximal exercise in male rowers [27]. This effect, however, was short lived as ghrelin levels returned to baseline after 30 minutes [27]. In a cycling study, ghrelin levels rose during low intensity exercise but did not rise with high intensity exercise [28]. The conflicting results and differences in protocols make it difficult for a consensus to be reached.

The literature on the effects of chronic exercise on ghrelin levels, however, is more clear. Previous studies have shown that weight loss resulting from reduced calorie intake increases ghrelin levels [5, 15]. This finding led researchers to examine whether an exercise-induced calorie deficit would lead to similar results. Multiple studies have examined changes in ghrelin with chronic exercise. A three month diet and exercise intervention produced significant increases in baseline ghrelin, lunch peak, dinner peak, nocturnal rise, and nocturnal peak [31]. A one-year aerobic exercise intervention found that exercise-induced weight loss, in the absence of reduced food intake, was associated with significant increases in ghrelin [25]. These increases in ghrelin were commensurate with the amount of weight lost [25]. In the exercise group,

however, ghrelin levels did not increase in those who did not lose weight or lost a mild amount of weight (0.5-3 kg) suggesting that there was no independent effect of exercise.

Another three month exercise intervention reported a significant increase in ghrelin in the weight loss group compared to the controls and the weight stable group [6]. By including a weight stable exercise group, this study was able to demonstrate that exercise training in the absence of weight loss had no effect on ghrelin. These studies add to the body of literature that suggests that ghrelin is a long-term regulator of energy balance. However, since many of these studies include weight loss as a function of the exercise protocols, it is difficult to determine if changes in ghrelin are related to weight loss or to physical activity itself.

Ghrelin, Fitness, and BMI

The results of studies on exercise and ghrelin raise important questions about the relationship between ghrelin, fitness status, and BMI. Ghrelin is associated with key components of energy balance [6]. For instance, ghrelin is negatively correlated with percentage of body fat, fat mass [14], waist circumference [32], BMI [32, 33], body weight, insulin and leptin [33]. The one year exercise induced weight loss intervention conducted by Foster-Schubert found that as body composition variables changed, the magnitude of the increase in ghrelin was correlated with the magnitude of the decrease in body weight, BMI, waist circumference, and total fat mass. The magnitude of the increase in ghrelin was not significantly correlated with the changes in lean body mass, intraabdominal fat, subcutaneous fat, energy intake, $VO_2\max$,

leptin or insulin [25]. One study found lower resting metabolic rate (RMR) to be associated with higher ghrelin levels in 63 young non-obese women [34]. Another study involving 65 non-obese women found that ghrelin was negatively correlated with RMR and thermic effect of food (TEF) independent of variations in body composition, insulin levels and daily energy intake [1]. In contrast to previous studies, however, no significant correlations were found between ghrelin and percent body fat, total fat mass, fat-free mass, physical activity, $VO_2\max$ or dietary disinhibition, dietary restraint, or feelings of general hunger [1]. Although no significant associations were found between ghrelin and $VO_2\max$ by St-Pierre et al., this area deserves more attention since the lack of significant findings could be attributed to the homogenous sample. The subject population consisted of 65 normal weight females. Another limitation may have been the measurement of only fasting ghrelin. Our current study included appetite measures, body composition measures, and fitness measures ($VO_2\max$) in relation to ghrelin and also included both genders and both fasting and fed ghrelin. These features distinguish it from previous studies in this area.

Ghrelin and Gender

A study examining plasma ghrelin and its relation to body composition reported that there were gender differences in the nature of these associations. Plasma ghrelin levels were inversely associated with fat mass measures in both males and females but there were gender differences in which of these body composition variables were related to ghrelin. For example, in males, plasma ghrelin was inversely correlated with abdominal fat mass and fat distribution index (calculated as the

trunk/leg fat mass ratio from dual energy x-ray absorptiometry body composition analysis). In females, inverse correlations were found between plasma ghrelin and BMI, total fat mass, and fat mass/lean mass ratio [35].

Since there are fluctuations in appetite with the menstrual cycle some studies have investigated if ghrelin levels also vary throughout the menstrual cycle. One study aimed specifically at abnormal menstrual cycles found that fasting ghrelin levels were at least 85% higher in women with exercise associated amenorrhea [36]. This supports the role for ghrelin as a metabolic indicator of chronic energy deficient states. In a normal menstrual cycle, however, a recent study reported that there were no significant changes in ghrelin throughout the cycle [37]. This is useful in planning future studies involving female subjects.

Fitness, Appetite, and Energy Intake

The relationship between fitness, appetite and energy intake also warrants further investigation. Appetite plays a central role in the regulation of energy balance [38]. The effects of exercise on appetite and food intake are controversial and depend on the intensity and duration of exercise. Several studies have investigated the effects of acute exercise on appetite and energy intake. There appears to be a weak coupling between energy expenditure and energy intake in the short term [39].

The effects of exercise on appetite and energy intake are influenced by the intensity of exercise performed. High intensity exercise has been shown to suppress hunger in the short term [39]. A study involving cycling at 35% and 68% of VO_{2max} indicated that hunger ratings were significantly lower in subjects in the highest

intensity exercise condition compared to those in the resting condition [40]. This hunger suppression did not translate to a reduction in food intake, however. The absence of an effect on food intake has been reported elsewhere [39, 41-43]. This is not surprising given that appetite is mediated by hormonal, psychological, and environmental factors [44]. Low to moderate intensity exercise has not been associated with this suppression [45]. This suggests that the intensity of the exercise has an effect on the extent of appetite and food intake suppression.

Exercise duration also has an effect on appetite and food intake. Erdmann et al. (2007) found that short duration exercise of 30-60 minutes had no effect on hunger/satiety sensations and subsequent food intake [28]. In this same study, a longer duration of exercise of 120 minutes produced an increase in food intake without having an effect on subjective hunger sensations.

Most studies dealing with exercise and hunger suppression have focused on aerobic exercise [8]. Little is known about the effects of resistance exercise on appetite. One recent study conducted by Broom et al. (2009) compared the effects of resistance and aerobic exercise on both hunger and ghrelin levels. Both hunger and ghrelin were significantly suppressed in the aerobic and resistance conditions compared to the control condition. This suppression was observed both during and after exercise until the first test meal (t=2 hours). After the consumption of the test meal, there were no significant differences in hunger between the exercise conditions and the control [30]. The finding that hunger is suppressed immediately after vigorous aerobic exercise (greater than 60% VO_{2max}) is consistent with previous studies including a prior study conducted by Broom involving running for 60 minutes at 72%

VO_2max [46]. The effects of resistance exercise on hunger were new findings.

Although resistance exercise was not found to suppress hunger to the same extent as aerobic exercise did, the suppression was still significant compared to the control. This attenuated response may be attributed to the lower energy expenditure along with the intermittent nature of resistance exercise compared to aerobic exercise [30].

Some studies have examined the effects of body weight, exercise and food intake. Non-obese women had lower post-exercise energy intakes after strenuous exercise, whereas obese women showed no change in intake across exercise conditions [41]. A different study reported no significant differences in post-exercise energy intake between obese and lean individuals [47]. However, obese subjects were found to have a decrease in hunger and appetite after exercise but without an accompanying decrease in energy intake [47]. Another study found that energy intake was higher in obese women compared to normal weight women after moderate intensity exercise and on the control day [44]. These results demonstrate the complexity of the relationship between exercise, body weight, appetite, and food intake.

While there is evidence of exercise-induced anorexia in the short term [45], a positive relationship exists between physical activity and energy intake in the long term [48]. It has been suggested that physiological and behavioral adaptations occur in the interim [48]. Short term (1-2 days) and medium term (7-16 days) exercise-induced negative energy balances of up to 4 MJ/d occur before compensation in energy intake is observed [45]. Beyond this, a compensatory effect takes place and food intake is increased to compensate for an average of approximately 30% of energy expended in

physical activity [45]. This compensation is not universally observed. Subjects can be classified as “compensators,” those who display increases in energy intake with exercise, or “non-compensators,” those who do not show increases in energy intake with exercise [45]. Further research is needed to determine the mechanisms through which this compensation occurs but there is some evidence that body composition plays a role in the extent of this compensation [49]. It has been suggested that fat mass acts as an energy buffer and increases in energy intake are not observed until lean body mass is threatened by the energy deficit created from physical activity [45].

A recent review analyzed the effects of chronic exercise on energy intake, energy balance and body weight [50]. While short to medium term exercise is effective at inducing a negative energy balance, long term exercise unless accompanied by calorie restriction, results in only minor weight loss.

The literature highlights many factors that could be responsible for the lack of a substantial effect of exercise on weight loss. These include both physiological and behavioral adaptations to counteract the new energy balance. Weight loss is associated with a compensatory reduction in total BMR [48] and an increase in ghrelin levels [5-7]. Also, as VO_2 max increases with exercise training, a lower energy expenditure is achieved for the same volume of exercise. Energy expenditure from exercise is also reduced with a lower body weight [48].

Behavioral mechanisms such as compensatory increases in energy intake from a less stringent dietary regimen may also counteract the energy deficit created by exercise. This is linked to the popular belief that the energy cost of exercise can offset the consumption of more energy dense foods [51]. Despite these compensations,

exercise is essential in preventing weight gain or regain in the long term [50]. The role of exercise in weight maintenance is thought to be a result of an improvement in appetite control and a better coupling between energy intake and energy expenditure [50]. One possible explanation for this improvement in appetite control may be linked to an increase in satiety hormones including polypeptide YY, glucagon-like peptide-1 and pancreatic polypeptide with exercise [52]. However, the extent to which changes in ghrelin may play a role in these relationships remains to be elucidated. The increase in these hormones may be responsible for the previously described phenomenon of exercise-induced anorexia.

Eating Behaviors and Appetite Control

Eating behavior is influenced by a multitude of factors ranging from physiological to cognitive and socio-cultural factors [53]. In order to fully understand eating behavior, it is important to examine both the physiological and non-physiological factors that affect energy intake. Physiological components of total food intake include hunger, satiation, satiety, and sensory-specific satiety. These factors are regulated by a combination of physical factors including gastric filling, distention, contraction and emptying and metabolic factors including gut peptides, neuropeptides, hormones and the detection of nutrients absorbed into the circulation [38].

At the physiological level, appetite and food intake are regulated by the brain and by hormones from the gastrointestinal tract, pancreas, adrenal glands and adipose tissue. The hypothalamus is involved in the central regulation of appetite and continuously receives neural, metabolic, and endocrine signals from the periphery.

Specifically, the arcuate nucleus within the hypothalamus contains many of the receptors for hormones and peptides known to be involved in the regulation of energy intake [50].

Traditional physiologic appetite theories focus on the individual's internal state and explain meal initiation by a "hunger drive" that increases proportionately to individual needs and is decreased by food intake [53]. Eating behavior, however, is much more complex than simple depletion-repletion models can explain and non-physiological factors must be considered. Appetite is influenced by a combination of cognitive, hedonic, psychological, habitual, cultural and social factors [38]. Physiological mechanisms that control food intake can easily be overridden by social and environmental factors.

Dietary Restraint and Disinhibition

The development of the Three Factor Eating Questionnaire (TFEQ) by Stunkard and Messick in 1985 has led to much research on eating behavior. The TFEQ measures three factors: restraint, disinhibition and hunger. The concept of dietary restraint has dominated the literature due to its relation to eating disorders, weight gain, and weight loss and dieting success [54]. Dietary restraint is a common control mechanism to lose weight or to prevent weight gain. A significant percentage of the population in Western countries consciously limits their food intake as a weight control strategy [55]. Dietary restraint is defined as a self-imposed resistance to the internal and external cues that regulate eating behavior [56]. Excessive restriction may have a counterproductive effect and eventually may lead to weight gain. Dietary

restraint is frequently associated with disinhibition and can even lead to disordered eating patterns [53].

Restrained eating behavior has also been linked to both metabolic and endocrine abnormalities [55]. A recent study measured the impact of dietary restraint on the plasma levels of different metabolites after the consumption of a standardized test meal in normal weight adults [55]. Restrained eaters were found to have significantly lower fasting insulin levels and lower insulin and glucose levels in the postprandial state. Restrained eaters also had better insulin sensitivity. Previous studies have demonstrated that restrained eaters have a reduced secretion of noradrenaline in the postprandial state [57] and a lower diet-induced thermogenesis [58] and fat oxidation [59] after a meal. The lower plasma insulin observed in restrained eaters is thought to be mediated by the reduced secretion of noradrenaline. Noradrenaline triggers the release of glucose into the bloodstream. Reduced secretion of noradrenaline would therefore result in lower levels of blood glucose in the postprandial state and would require less insulin [55].

A longitudinal study examined cognitive restraint and weight gain in a large sample of normal and overweight adults and adolescents [60]. The findings indicated that restraint was positively correlated with BMI in normal weight but not in overweight subjects. Restrained eating was not predictive of weight gain. However, a high initial BMI was associated with a greater increase in cognitive restraint over the two year period. These findings were in contrast to a previous cross-sectional study performed on restrained eating and weight gain that reported that restrained eating is higher with greater BMI [61].

Although most of the literature has focused on restraint, disinhibition also plays an important role in appetite and weight regulation. Disinhibition refers to a tendency towards over-eating. Disinhibition includes over-eating because others are eating, not being able to resist food, eating in response to negative mood states, and over-eating highly palatable foods [62]. Disinhibition is positively correlated with BMI and obesity [62]. It is also predictive of less successful weight loss attempts and of weight regain. In addition, disinhibition is associated with lower self-esteem, poor psychological health, and low physical activity [62].

Relatively little research has directly examined the relationship between disinhibition and physical activity but a few studies have been conducted. One study suggested that women who exercised and had high disinhibition scores increased their energy intakes leading to a positive energy balance [59]. On the other hand, acute exercise was shown to have a beneficial effect on women who had high disinhibition scores by decreasing their motivation to eat and increasing their preference for low-fat foods[54].

Effects of Dietary Restraint and Exercise on Appetite Control

Exercise has been shown to be more effective than dieting in inducing a negative energy balance in the short term [51]. A study that compared the effects of a diet induced negative energy balance to an exercise induced negative energy balance found that hunger ratings and food cravings were significantly higher in the diet condition [51]. Another study that examined the impact of dietary restraint and a single bout of moderate-intensity exercise on post-exercise energy intake in sedentary

males found that there was not a significant effect of exercise on energy intake either immediately after exercise or over a twelve hour time period [63]. The authors expected to find the overweight males with high restraint to become disinhibited by exercise and have significantly higher energy intakes but this was not observed. This could be attributed to the fact that increased energy expenditure from physical activity is not immediately compensated for by increases in energy expenditure [48]. A study on exercise, dietary restraint and energy intake in females who had recently lost weight found that exercise did not result in a significant increase in energy intake. This was not surprising since the majority of the subjects were classified as highly restrained. The subjects were also categorized into overeaters or undereaters depending on whether or not their self-selected energy intake levels exceeded their maintenance requirements. Compared to the undereaters, the overeaters had higher disinhibition scores [64].

Queen's College Step Test

Maximum oxygen uptake ($VO_2\max$) is a widely accepted measure of cardiorespiratory fitness. The experimental protocol used to determine $VO_2\max$, however, is difficult, exhausting, and requires a well-equipped laboratory [65]. These factors have led to the development of simpler techniques to predict $VO_2\max$. The Queen's College Step test is a frequently used 3 minute sub-maximal technique to estimate $VO_2\max$ [66]. Since the development of this indirect method of measuring $VO_2\max$, studies have assessed its validity in different populations. The Queen's College step test was validated in male college students in India [67] but was found to

be inaccurate for estimation in female college students in India [65]. Our analysis estimated $VO_2\text{max}$ using a new equation for males that was recently developed at the University of Rhode Island. This equation was developed to more accurately estimate $VO_2\text{max}$ in males using heart rate and weight (Deborah Riebe, personal communication).

Conclusion

Since ghrelin's discovery in 1999 it has been studied extensively. Research has centered on its roles in appetite regulation, energy intake, and energy balance. Weight loss studies have demonstrated that ghrelin levels fluctuate with body weight. This along with the prevalence of obesity has highlighted the importance of research focused on body weight regulation on a hormonal level. Recent research has also explored the relationship between ghrelin and energy expenditure. The differences in type, duration, and intensity of the exercise protocols have made it difficult for a consensus to be made on the effects of exercise on ghrelin. Future studies in this area will help researchers understand the underlying mechanisms responsible for ghrelin's roles in both energy intake and energy expenditure. This present study will help to clarify previous findings and provide a better understanding of how fitness status and BMI relate to ghrelin and appetite.

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APPENDIX B

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

No vigorous physical activities → **Skip to question 3**

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

No moderate physical activities → **Skip to question 5**

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

No walking → **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

APPENDIX C

Three-Factor Eating Questionnaire

For the true/false items on the next two pages, indicate whether or not you feel or behave that way by selecting whether the statement is TRUE or FALSE for you by carefully placing a check (✓) in the box that represents either true or false.

	TRUE	FALSE
1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to stop from eating, even if I have just finished a meal.		
2. I usually eat too much at social occasions, like parties and picnics.		
3. I am usually so hungry that I eat more than three times a day.		
4. When I have eaten my quota of calories, I am usually good about not eating any more.		
5. Dieting is so hard for me because I just get too hungry.		
6. I deliberately take small helpings as a means of controlling my weight.		
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.		
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.		
9. When I feel anxious, I find myself eating.		
10. Life is too short to worry about dieting.		
11. Since my weight goes up and down, I have gone on reducing diets more than once.		
12. I often feel so hungry that I just have to eat something.		
13. When I am with someone who is overeating, I usually overeat too.		
14. I have a pretty good idea of the number of calories in common food.		
15. Sometimes when I start eating, I just can't seem to stop.		
16. It is not difficult for me to leave something on my plate.		
17. At certain times of the day, I get hungry because I have gotten used to eating then.		

	TRUE	FALSE
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.		
19. Being with someone who is eating often makes me hungry enough to eat also.		
20. When I feel blue, I often overeat.		
21. I enjoy eating too much to spoil it by counting calories or watching my weight.		
22. When I see a real delicacy, I often get so hungry that I have to eat it right away.		
23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.		
24. I get so hungry that my stomach often feels like a bottomless pit.		
25. My weight has hardly changed at all in the last ten years.		
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.		
27. When I feel lonely, I console myself by eating.		
28. I consciously hold back at meals in order not to gain weight.		
29. I sometimes get very hungry late in the evening or at night.		
30. I eat anything I want, any time I want.		
31. Without even thinking about it, I take a long time to eat.		
32. I count calories as a conscious means of controlling my weight.		
33. I do not eat some foods because they make me fat.		
34. I am always hungry enough to eat at any time.		
35. I pay a great deal of attention to changes in my figure.		
36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.		

Please answer the questions in this section by circling the number above the response that is appropriate for you.

37. How often are you dieting in a conscious effort to control your weight?

1	2	3	4
rarely	sometimes	usually	always

38. Would a weight fluctuation of 5 lbs affect the way you live your life?

1	2	3	4
not at all	slightly	moderately	very much

39. How often do you feel hungry?

1	2	3	4
only at mealtimes	sometimes between meals	often between meals	almost always

40. Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4
never	rarely	often	always

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	3	4
easy	slightly difficult	moderately difficult	very difficult

42. How conscious are you of what you are eating?

1	2	3	4
not at all	slightly	moderately	extremely

43. How frequently do you avoid "stocking up" on tempting foods?

1	2	3	4
almost never	seldom	usually	almost always

44. How likely are you to shop for low calorie foods?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

45. Do you eat sensibly in front of others and splurge alone?

- | | | | |
|------------|-------------|------------|-------------|
| 1
never | 2
rarely | 3
often | 4
always |
|------------|-------------|------------|-------------|

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

- | | | | |
|---------------|-------------------------|---------------------------|---------------------|
| 1
unlikely | 2
slightly
likely | 3
moderately
likely | 4
very
likely |
|---------------|-------------------------|---------------------------|---------------------|

47. How frequently do you skip dessert because you are no longer hungry?

- | | | | |
|----------------------|-------------|------------------------------|--------------------------|
| 1
almost
never | 2
seldom | 3
at least
once a week | 4
almost
every day |
|----------------------|-------------|------------------------------|--------------------------|

48. How likely are you to consciously eat less than you want?

- | | | | |
|---------------|-------------------------|---------------------------|---------------------|
| 1
unlikely | 2
slightly
likely | 3
moderately
likely | 4
very
likely |
|---------------|-------------------------|---------------------------|---------------------|

49. Do you go on eating binges though you are not hungry?

- | | | | |
|------------|-------------|----------------|------------------------------|
| 1
never | 2
rarely | 3
sometimes | 4
at least
once a week |
|------------|-------------|----------------|------------------------------|

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself? Please circle your answer.

0--eat whatever you want, whenever you want it

1--usually eat whatever you want, whenever you want it

2--often eat whatever you want, whenever you want it

3--often limit food intake, but often 'give in'

4--usually limit food intake, rarely 'give in'

5--constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behavior? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1
not like me

2
little like
me

3
pretty good
description of me

4
describes me
perfectly

APPENDIX D

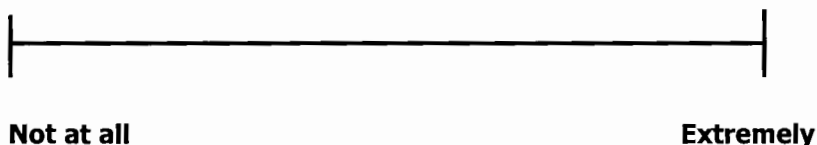
Energy Metabolism Laboratory Satiety Rating Scale

Subject #	Test Condition	Visit #	Visit Date

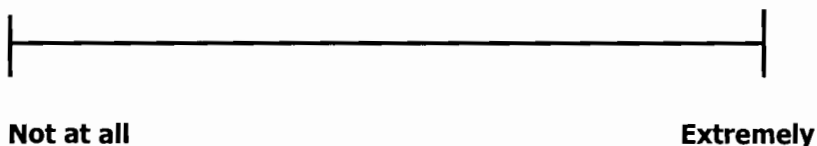
Please answer each question by placing a small vertical hash (“ | ”) across the horizontal line at the point that best reflects your present feelings.

Clock Time: _____

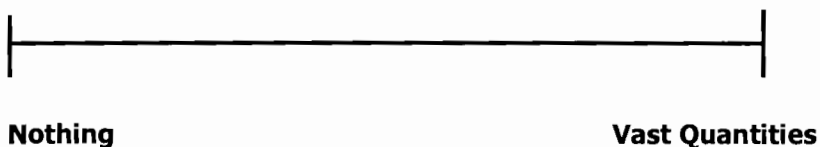
1. How hungry are you right now?



2. How satisfied (satiated) are you right now?



3. How much could you eat right now?



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