

Influence of Aerobic Exercise on Appetite-Regulating Hormones, Ghrelin-o-Acyltransferase and Perceived Hunger in Normal Weight and Obese Adults

Michael Bruneau Jr^{1*}, Richard Wood², Susan Sotir³, Samuel Headley⁴, Elizabeth O'Neil⁴, Juan Muñiz⁵, May Cheung⁶, Marissa Pontarelli⁷, Susan Lachowski⁸, Vincent Paolone⁹

¹Department of Health Sciences, Drexel University, 60 N 36th Street, Philadelphia PA, USA

²Clinical Product Development, CVS Health, Woonsocket, RI, USA

³Breakthrough Performance Coaching, LLC, Ipswich, MA, USA

⁴Department of Exercise Science and Athletic Training, Springfield College, Springfield, MA, USA

⁵Department of Nutrition Sciences, Drexel University, Philadelphia, PA, USA

⁶Department of Health and Nutrition Sciences, Brooklyn College, Brooklyn, NY, USA

⁷Department of Medical Oncology, Thomas Jefferson University Hospital, Philadelphia, PA, USA

⁸Department of Exercise Science, American International College, Springfield, MA, USA

⁹Department of Exercise Science and Athletic Training, Springfield College, Springfield, MA, USA

Corresponding Author: Michael Bruneau Jr, E-mail: mlb425@drexel.edu

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ABSTRACT

Background: Obesity is a major public health issue in the United States (U.S.), affecting an estimated 78 million US adults. Aerobic exercise (AE) is recommended by the American College of Sports Medicine to prevent and treat obesity, yet the effects of AE on circulating hunger hormones including acylated ghrelin and its biological catalyst, ghrelin o-acyltransferase (GOAT) are less known. **Objectives:** We investigated the effects of AE on circulating concentrations of appetite regulating hormones and GOAT in a pilot sample of adults classified with normal weight (NW) and obese (OB) body weight status. **Methods:** Using a quasi-experimental design, nine adults with NW (n=4, body mass index [BMI] = 21.3±1.2 kg/m²) and OB (n=5, BMI = 38.9±6.2 kg/m²) body weight status completed a preliminary health/fitness assessment. Participants returned to the laboratory on three separate occasions, separated by ≥ 48 hours to perform cycle exercise at 30% and 60% oxygen uptake reserve (VO₂R) or a seated control session with no exercise for 40 min. Fifteen mL of blood was taken pre-and-post exercise and control and were assayed in duplicate. Nonparametric procedures determined whether mean rank differences existed between NW and OB for acylated ghrelin, leptin, insulin, and GOAT in response to exercise and control. Alpha levels were set *a priori* to $p < 0.05$. **Results:** Significant mean rank reductions were found in GOAT after compared to before AE and control for NW and OB ($p < .05$). Significant mean rank differences were found in acylated ghrelin after compared to before performing AE at 60% VO₂R in NW and OB ($p < .05$); however, differences were not observed between NW and OB ($p > .05$). **Conclusions:** Our findings reveal the first available data regarding the effects of AE on GOAT, with NW and OB experiencing equivocal changes pre-to-post AE at 60% VO₂R, and in response to a seated control session.

Key words: Cardiometabolic Risk Factors, Exercise, Appetite Regulation, Hunger, Obesity

INTRODUCTION

Obesity is a major public health issue in the United States (U.S.). Obesity, defined as a body mass index (BMI) ≥ 30 kg/m² affects an estimated 100.1 million U.S. adults (41.9%) each year (Hales et al., 2017; Ogden et al., 2014). The prevalence of obesity is higher in women (41.1%) compared to men (37.9%) and among non-Hispanic black (46.8%) and Hispanic (47.0%) adults compared to non-Hispanic white (37.9%) and Asian (12.7%) adults. Obesity is a major independent risk factor for cardiovascular disease (CVD), type II

diabetes mellitus (T2DM), and cancer (Tiryaki-Sonmez et al., 2013). The total medical costs attributed to obesity now exceed \$260.6 billion per year, and accounts for approximately 1 out of every 10 U.S. healthcare dollars spent (Cawley et al., 2021). Due to the significant impact of obesity on U.S. public health, the prevention, treatment, and management of obesity are major public health priorities.

Aerobic exercise (AE) is an effective lifestyle therapy for preventing, treating, and managing overweight and obesity. The National Institutes of Health reported that a 5% to 10%

reduction in body weight could provide significant health benefits among overweight and obese adults (NHLBI Obesity Education Initiative Expert Panel on the Identification, 1998). For this reason, adults who are overweight or obese are encouraged to exercise in accordance with the American College of Sports Medicine (ACSM) recommendations that involve the performance of moderately intense AE for 30 to 60 min on most, preferably all days of the week (American College of Sports Medicine, 2022). However, and despite these recommendations, the effect of exercise on circulating appetite-regulating hormones are less known. Ghrelin is a potent orexigenic peptide hormone secreted from the submucosa of the gastric fundus during periods of negative energy balance (Borer, 2013; Gueugnon et al., 2012; Kirchner et al., 2012; Seoane et al., 2012). Acylated ghrelin, considered the active form of ghrelin, is catalyzed via the ghrelin-o-acyltransferase enzyme (GOAT). Leptin and insulin are potent anorexigenic peptide hormones secreted from adipose tissue and pancreatic beta cells during periods of positive energy balance. Together, these appetite-regulating hormones function to bind to differentiated target receptors in the ventromedial hypothalamus to signal the enhancement or suppression of hunger (Borer, 2013). Although ghrelin, leptin and insulin collectively function to bind to differentiated target receptors, researchers have reported metabolism and interactions of these hormones to differ between adults living with normal weight (NW) and obese (OB) body weight statuses (Considine, 1996; King, 2013; Makris, Alexandrou, Papatsoutsos, Malietzis, Tsilimigras, Guerron, Moris, 2017; Modan, 1985; Obradovic, Sudar-Milovanovic, Soskic, Essack, Arya, Stewart, Gojbori, Isenovic, 2021; Wondmkun, 2020). Among adults with a NW body weight status, ghrelin peaks before and plummets after food ingestion, whereas leptin and insulin peak after and plummet before food ingestion (King, 2013). Among adults with a OB body weight status, ghrelin, leptin, and insulin concentrations remain elevated despite food ingestion, perhaps due to impaired leptin and/or insulin signaling or leptin and/or insulin resistance (Considine, 1996; King, 2013; Makris et al., 2017; Modan, 1985; Obradovic et al., 2021; Wondmkun, 2020).

To date, several researchers have attempted to explore the influence of AE on circulating ghrelin, leptin, and insulin in adults with NW body weight status (Bogardus, 1988; Bouassida, 2006; Broom, 2007; Essig, 2000; King, 2011; Olive, 2001; Perusse, 1997; Romjin, 1993; Schmidt, 2004; Weltman, 2000); but to the best of our knowledge, none have attempted to explore the influence of exercise on GOAT in adults with an overweight and OB body weight status (Colberg, 1996; Minuk, 1981; Tiryaki-Sonmez et al., 2013). Furthermore, researchers conducting these studies often: (a) recruited small and homogeneous samples; (b) implemented exercise interventions that varied in frequency, intensity, time, and type (FITT); (c) recruited samples subjected to selection bias; (d) explored ghrelin without examining the influence of GOAT; and (e) neglected to explore possible confounders which might impact the influence of exercise on circulating hunger hormones and GOAT among NW and OB adults. Accordingly, we examined the effects of acute

AE on circulating appetite-regulating hormones and GOAT in a sample of adults with NW and OB body weight statuses in a pilot study. We hypothesized that 1) those classified with an OB body weight status would have similar pre-to-post exercise and control circulating GOAT, and 2) those classified with a NW body weight status would have a reduction of appetite-regulating hormones and GOAT post light and vigorously intense exercise compared to a seated control session with no exercise.

METHODS

Study Design

The Institutional Review Board at Springfield College approved all testing procedures prior to any data collection in accordance with the Declaration of Helinski. To conduct our pilot study, we attempted to determine the acute effects of AE on circulating acylated ghrelin, leptin, insulin, and GOAT via a quasi-experimental design and included cycle exercise performed at 30% and 60% of the maximum oxygen uptake reserve (VO_{2R}) and a seated control session with no exercise for 40 min. Appetite-regulating hormones and GOAT were measured pre-and-post exercise and in the control condition.

Participants

A preliminary power analysis for each dependent variable was performed with G*Power (Faul, 2007) version 3.1 to detect a medium to large effect size of exercise-induced changes in appetite-regulating hormones, GOAT and perceived hunger with 80% power. We determined that a pilot sample of nine participants would yield 84% power to detect an effect size of 0.50. Thus, adults classified with a NW ($n=4$) and OB ($n=5$) body weight status and who were sedentary and between 18-55 years of age from Western Massachusetts were recruited with flyers and letters for the study. The participants were classified as either NW or OB from BMI, waist circumference, and Bod Pod measurements. Participants classified as NW had a BMI between 18.5 – 24.9 kg/m^2 , a waist circumference < 80.0 cm (31.5 in) for men and < 70.0 cm (27.5 in) for women and an age-adjusted body fat percentage between 10.0% and 19.9% for men and 20.0% and 29.9% for women. Participants classified as OB had a BMI ≥ 30 kg/m^2 , a waist circumference > 102.0 cm (40.0 in) for men and > 88.0 cm (34.6 in) for women, and an age-adjusted body fat percentage > 25.0% for men and > 39.0% for women. Participants were considered sedentary when not performing at least 30 min of moderate physical activity on at least three days per week for the past three months. Any participant with known or suggestive cardiovascular, pulmonary, or metabolic disease or with musculoskeletal injury was excluded from the study. Similarly, participants prescribed and/or taking cardiovascular, vasodilatory, antilipemic, blood modifying, hormonal, adrenal, androgenic-anabolic, contraceptive, thyroid, antidiabetic, or weight reduction agents were excluded from the study. All participants completed an informed consent, a physical activity readiness questionnaire (PAR-Q) (Warburton, 2011) a

preparticipation screening questionnaire (Balady, 1998) and a medical history form prior to enrollment.

Measurement Instruments

The following measurement instruments were used to complete the study: Paffenbarger Physical Activity Questionnaire (PPAQ), Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), hunger scale, BMI, waist circumference, Bod Pod, YMCA submaximal exercise Test (YSET), and biological assays. The PPAQ, PSS, PSQI, BMI, waist circumference, Bod Pod and YSET were measured at the baseline orientation session. Hunger scales and biochemical assays were measured pre-and-post exercise and control at the exercise and control sessions.

Paffenbarger physical activity questionnaire

The PPAQ was used to assess habitual physical activity among participants, including informal activities of daily living (e.g., city blocks walked, stairs climbed), leisure-time activities, and formal exercise (Paffenbarger et al., 1978). Activity scores in kcal per week were calculated based on sums of calories expended across durations and intensities of different activities. The PPAQ has excellent reliability (Pereira, 1997) and predictive validity (Harris, 1994) with correlation coefficients between 0.30 and 0.73 and -0.13 and 0.69 for reliability and validity. The PPAQ assesses global activity over the past month and is sensitive to changes during interventions (Jeffery, 1998).

Perceived stress scale

The PSS was used to assess the degree of perceived stress among participants over the past month (Cohen, 1983). PSS scores were determined by reversing the responses to items four, five, seven and eight and then summing across all scaled items (Cohen, 1983). The PSS has good predictive validity and test-retest reliability with correlation coefficients of $r = 0.76$ and $r = 0.85$ for validity and reliability (Cohen, 1983).

Pittsburgh sleep quality index

The PSQI was used to assess the sleep habits of subjects over the past month (Buysse et al., 1989). PSQI scores were computed by summing the composite scores for sleep duration, sleep disturbance, sleep latency, day dysfunction from sleepiness, sleep efficiency, overall sleep quality, and the need of medication to sleep. The PSQI has excellent construct validity and test-retest reliability with a correlation coefficient of $r = 0.85$ for reliability and a significant *Student-Newman-Kuel* statistic for validity ($F = 45.1^4, p = 0.001$) (Buysse et al., 1989).

Hunger scale

The rating of perceived hunger measures perceived hunger with a visual hunger scale, ranging from 0 to 16. Higher hunger scores indicate greater hunger (Broom, 2007). To assess perceived hunger, the participant points to a number on the

hunger scale that best represented the amount of hunger felt. The hunger scale includes the phrases “not hungry”, “fairly hungry”, “hungry”, and “very hungry”. The hunger scale has excellent concurrent validity as evidenced by identical responses to analogue scales developed by King, Burley, and Blundell (King, 1994) and Broom (Broom, 2007; Burns, 2007; King, 1996).

Body mass index and waist circumference

BMI was calculated from height (assessed in stocking feet and measured to the nearest 0.10 cm) and weight (assessed in stocking feet with excessive clothing and materials removed and measured to the nearest 0.10 kg) with a professional scale (BodPod®, COSMED USA, Chicago, IL). BMI was computed as weight in kg/height in m² (American College of Sports Medicine, 2022). Waist circumference was measured at the height of the iliac crest with a Gulick tape measure (Sammons Preston, Chicago, IL) to the nearest cm (Lohman, 1988).

Air displacement plethysmography

The Bod Pod (COSMED USA, Chicago, IL) was used to measure body density via air displacement plethysmography. Air displacement plethysmography measures changes in air volume within the chamber according to Boyle’s law. The volume of air displaced within the chamber is equivalent to body volume and is computed as the difference between the volume of air remaining in the chamber (with the participant) and the volume of air in the chamber when empty (without the participant), (Heyward, 2010) body fat percentage was predicted from body density with population specific two-component formulae developed by Heyward and Wagner (Heyward, 2004). The validity and reliability of the Bod Pod measurement is good with documented intra-tester coefficients of variation between 1.7% and 4.3% and standard error of measurements between 1.8% and 3.7% (Ball, 2004; Ballard, 2004; Collins, 1999; Fields, 2002).

YMCA sub-maximal ergometer test

The YMCA Sub-maximal Ergometer Test (YSET) is used to estimate cardiorespiratory exercise capacity (American College of Sports Medicine, 2022). The YSET is a multistage cardiorespiratory measure beginning with an initial 2 min warm-up period of 0 kgm resistance on a cycle ergometer (Monark ergometric 818, Stockholm, Sweden). Following the initial warm-up, 150 kgm workload was applied for 3 min, representing stage one. All subsequent stages were progressed incrementally in 3 min intervals based off the heart rate (HR) values observed in the prior stage. HR was measured in beats per min (bpm) and recorded during the 2nd and 3rd min of each stage using a Polar Heart Rate monitor, model 190027142 (Polar Electro Oy, Kempele, Finland). Blood pressure (BP) and rating of perceived exertion (RPE) were also monitored near the end of the 2nd and 3rd min of each stage with a Classic Hand Aneroid model sphygmomanometer (Tycos® Classic Hand Aneroids, Welch Allyn®,

Skaneateles Falls, NY) a Cardiology Stethoscope (3m TM Littman® Cardiology III Tm Stethoscope, 3 M TM, Littman® Stethoscopes, St. Paul, MN), and a Borg 6-20 scale (Borg, 1982). Peak cardiorespiratory capacity was computed using a YSET $\text{VO}_{2\text{max}}$ prediction equation. The YSET has good predictive validity of $\text{VO}_{2\text{max}}$ with correlation coefficients of $r = 0.77$ and $r = 0.79$ for treadmill and cycle max tests (Beekley, 2004).

Biochemical assays

Acylated ghrelin, leptin, insulin, and GOAT were measured with Biomatik® and ThermoFisher® enzyme-linked immunosorbent assays (ELISAs). ELISA is a biochemical assay that uses antibodies to detect a substrate of interest against the antigen for the antibody (Lequin, 2005). If interactions exist, a visible change in color appears. The visual change in color is assessed by an electronic plate reader, which determines the concentration of the substrate in blood serum. The validity and reliability of the ELISAs are dependent upon the standards set by the manufacturers (Biomatik® USA, LLC, 105-501 Silverside Rd, Wilmington, DE; ThermoFisher® Waltham, MA).

Procedures

Participants were scheduled for a total of four laboratory sessions. The first session served as a baseline orientation session. At the baseline orientation session, participants completed the PAR-Q (Warburton, 2011), AHA/ACSM Health/Fitness Preparticipation Screening Questionnaire (Balady, 1998), and medical history form for risk classification of cardiovascular disease (CVD). Participants classified as low risk (< 2 positive risk factors) were enrolled in the study. Participants classified as moderate risk (> 2 positive risk factors) were referred to a primary care physician to obtain medical clearance and were questioned about medication prescription or consumption that precluded enrollment in the study. Participants granted medical clearance and not prescribed or taking medications were enrolled in the study. Participants classified as high risk (known or suggestive cardiovascular, pulmonary, metabolic, or renal disease) were excluded from the study. In addition to completing the PAR-Q, AHA/ACSM Health/Fitness Preparticipation Screening Questionnaire and medical history forms, participants completed the PPAQ (Paffenbarger et al., 1978) to assess baseline physical activity, PSS (Cohen, 1983) to assess baseline stress, and the PSQI Index (Buysse et al., 1989) to assess baseline sleep quality. The Compendium of Physical Activities (Ainsworth et al., 2011) quantified light and vigorous sport or recreational activities for the PPAQ.

Upon completing the PAR-Q, AHA/ACSM Health/Fitness Preparticipation Screening Questionnaire, medical history form, PPAQ, PSS and PSQI, participants then underwent a comprehensive health fitness assessment to determine baseline BMI, waist circumference, body fat percentage and estimated $\text{VO}_{2\text{peak}}$. The body composition estimates were used to classify participants as either NW or OB. The YSET predicted $\text{VO}_{2\text{peak}}$ and was then used to determine

a relative exercise intensity of 30% (30 % $\text{VO}_{2\text{R}}$) and 60% (60% $\text{VO}_{2\text{R}}$) oxygen uptake reserve ($\text{VO}_{2\text{R}}$) and control (CONT). All participants were instructed to: (a) refrain from ingesting food, alcohol or caffeine or using tobacco products within three hours of testing; (b) avoid exercise and strenuous physical activities on testing session days; (c) wear comfortable and loose fitted clothing to permit freedom of movement and walking or running shoes; (d) arrange transport to and from the testing session; and (e) drink ample fluids over the 24 hr preceding exercise sessions. Following the baseline orientation session, participants were scheduled to return to the laboratory no sooner than 48 hr later. At session two, participants were randomly assigned to an acute bout of AE performed on a cycle ergometer at 30% or 60% $\text{VO}_{2\text{R}}$ for 40 min, or a seated control session with no exercise for 40 min. AE sessions were preceded by a 5 min warm-up period with no resistance on the cycle ergometer, and was followed by administration of an exercise intensity equated to 30% or 60% $\text{VO}_{2\text{R}}$. AE intensities were monitored and maintained at 30% or 60% $\text{VO}_{2\text{R}}$ with heart rate reserve (HRR) using the Karvonen method. Perceived hunger was assessed pre-and-post exercise and control after ≥ 5 min of steady state rest with a visual hunger scale (Broom, 2007).

Fifteen mL of blood was taken via venipuncture pre-and-post exercise and control after ≥ 5 min of seated rest. The venipuncture was performed by a researcher trained in blood processing procedures (RW). Of the 15 mL drawn for each time point, 5 mL was placed into a sodium citrate tube (Becton Dickson and Company, Franklin Lakes, NJ) and the remaining 10 mL was placed into a tube containing EDTA (Becton Dickson and Company, Franklin Lakes, NJ). All blood samples were centrifuged at 4°C and were spun at 1,500 x g for 15 min. The samples were then separated into plasma and sodium citrate 600 μL tubes for storage at -80°C until the blood processing laboratory analyzes were ready to be performed. The μL tubing was labeled for participant, assay type, and time point as a quality control measure.

After completing visit two, the participants once again returned to the laboratory ≥ 48 hr later for visit three. At visit three, participants once again were randomized to one of the two remaining treatment or control conditions for 40 min with pre-and-post blood draws following the same procedures as visit two. Visit four was then scheduled ≥ 48 hr later. At visit four, participants completed the remaining treatment or control condition for 40 min with pre-and-post blood draws following the same procedures as visits two and three, at which point the participant successfully completed the study protocol. After all participants completed all sessions of the study protocol, the blood samples were removed from storage and shaken (Brownstead International Model 4625) cleansed (Thermoelectron Corporation, Wellwash 4MK2, Fisher Scientific) and assayed (Dynex Magellen Biosciences MDX Revelation) in duplicate with standard Biomatik® and ThermoFisher® ELISA kits and quality controls. The Biomatik and ThermoFisher kits were customized by the manufacturer to detect acylated ghrelin, leptin insulin and GOAT concentrations in the blood samples collected.

Statistical Analyses

Descriptive statistics (means \pm standard deviations) were computed for all study variables. A 2 (group) X 3 (treatment) analysis of variance (ANOVA) was used to determine mean differences of acylated ghrelin, leptin, insulin, and GOAT in response to acute AE performed at 30% VO_2R , 60% VO_2R and a seated control session among NW and OB adults. Two independent variables, and four dependent variables were used to perform the ANOVA; however, non-parametric Wilcoxon Signed Rank and Mann-Whitney U-tests were performed to replace the ANOVA because the basic assumption of normality was violated.

The basic assumptions of the Wilcoxon Signed Rank and Mann-Whitney Utests include: (a) independent observations of the independent variable; (b) dependent observations of the dependent variable; (c) ordinal, interval, or ratio level of measurement of the dependent variables; (d) nominal or ordinal level of measurement of the independent variables; (e) equal distribution of scores; and (f) univariate normality/non-normality of the dependent variables. All specific aims and hypotheses were specified before data collection procedures proceeded and all statistical procedures were planned and pre-specified before conducting any analyses. Verification of all statistical basic assumptions and randomization procedures were performed with the Statistical Package for the Social Sciences (SPSS), version 27.0 and alpha levels were set *a priori* to $p < 0.05$.

RESULTS

To determine the influence of acute AE on the change in circulating concentrations of appetite-regulating hormones,

GOAT and perceived hunger between adults classified with a NW and OB body weight status, we used nonparametric procedures. Preliminary data screening procedures were performed to determine the accuracy, completeness, and normality/nonnormality of all data collected. All basic assumptions for these procedures were assessed and confirmed with no outliers or extreme scores detected. The following sections include: the descriptive and inferential outcomes for each appetite-regulating hormone, GOAT, and perceived hunger.

Participants

The study sample ($N=9$) included younger (33.2 ± 10.2 years) NW ($21.3 \pm 1.3 \text{ kg/m}^2$) and OB ($38.9 \pm 6.2 \text{ kg/m}^2$) women (55.6%) and men (44.4%) of white (88.9%) and Hispanic (11.1%) ethnicity. Descriptive characteristics for the total sample and by body weight classification are presented in Table 1.

Ghrelin-o-acyltransferase (GOAT)

Means and standard deviations of GOAT for the total sample and by body weight classification are presented in Table 2. Mann Whitney U-tests revealed no significant between-group mean rank difference at baseline ($p > .05$). Wilcoxon signed rank tests assessed within-group pre-to-post 40 minutes of cycling exercise at 30% VO_2R , at 60% VO_2R , and in the control condition. A significant pre-to-post difference in GOAT was found for the total sample after cycle exercise performed at 30% VO_2R ($z = -2.666$, $p = .008$) for 40 min, after completing cycle exercise performed at 60%

Table 1. Physical characteristics of the total sample and by body weight classification

	Total Sample	Normal Weight (N = 4)	Obese (N = 5)	P
Age (yrs)	33.2 \pm 10.2	28.5 \pm 9.6	37.0 \pm 10.0	0.24
Height (cm)	157.6 \pm 34.8	169.6 \pm 16.8	148.1 \pm 44.2	0.39
Wt (kg)	89.1 \pm 28.4	61.7 \pm 13.4	111.0 \pm 11.4	<0.001*
BMI (kg/m ²)	31.1 \pm 10.3	21.3 \pm 1.3	38.9 \pm 6.2	<0.001*
Waist Circum (cm)	90.7 \pm 21.4	69.0 \pm 5.8	108.0 \pm 6.8	<0.001*
Body Fat (%)	32.3 \pm 14.0	21.2 \pm 6.8	41.2 \pm 11.7	0.02*
SBP (mmHg)	117.2 \pm 10.1	110.8 \pm 7.6	122.4 \pm 9.1	0.08
DBP (mmHg)	74.9 \pm 13.8	64.3 \pm 13.5	83.4 \pm 6.4	0.03*
Sit Up (cm)	14.2 \pm 14.6	29.0 \pm 2.0	2.4 \pm 5.4	<0.001*
Push Up (reps)	7.2 \pm 9.7	13.0 \pm 11.6	2.6 \pm 5.3	0.11
Handgrip (kg)	61.5 \pm 18.3	58.0 \pm 22.1	64.3 \pm 16.7	0.64
Sit and Reach (cm)	36.0 \pm 17.5	47.5 \pm 10.7	26.8 \pm 17.1	0.07
VO_2 peak (ml/kg/min)	32.0 \pm 12.2	42.0 \pm 10.5	23.9 \pm 5.8	0.01*
PPAQ (kcal/wk)	2,020.6 \pm 3,022.1	2,175.10 \pm 2,408.0	1,897.1 \pm 3,724.4	0.90
PSS (0-40)	16.8 \pm 6.2	18.5 \pm 7.0	15.4 \pm 6.0	0.50
PSQI (0-21)	6.9 \pm 4.2	8.8 \pm 5.3	5.4 \pm 2.8	0.26

cm = centimeters, kcal/wk = kilocalories expended per week; kg = kilograms; kg/m² = kilograms per meter squared; ml/kg/min = milliliters of oxygen consumed per kilogram of body weight per minute; mmHg = millimeters of mercury; reps = number of push-ups performed; Yrs = years; % = percentage; * indicates a statistically significant difference between normal weight and obese adults in the study sample.

Table 2. Acylated ghrelin, leptin, insulin and ghrelin o-acyltransferase by body weight classification before and after exercise at 30% and 60% maximum oxygen uptake reserve and seated control

	30% VO ₂ R		60% VO ₂ R		Seated Control	
	Pre	Post	Pre	Post	Pre	Post
Acylated Ghrelin (pg/mL)						
Normal Weight	1,375.43±809.42	1,521.33±759.69	1,515.70±797.69	1,484.05±828.03	1,391.56±768.96	1,365.93±842.16
Obese	654.02±323.50	598.56±223.86	776.14±727.13	482.46±355.30	697.30±336.63	551.96±267.94
Leptin (pg/mL)						
Normal Weight	4,876.69±3,597.39	5,120.24±4,147.90	5,666.31±4669.75	4,880.96±3,185.09	7,006.68±6626.21	6,087.77±5,686.10
Obese	38,762.89±32,012.37	39,312.43±31,389.68	40,097.47±29,812.67	38,249.14±29,672.79	39,615.66±30,873.15	38,030.90±32,284.52
Insulin (mg/mL)						
Normal Weight	7.41±2.79	8.55±4.45	5.96±2.70	6.55±1.93	8.19±4.01	5.08±2.71
Obese	29.83±21.76	20.11±10.55	28.46±30.57	20.91±12.06	24.72±18.43	25.69±16.21
Ghrelin O-Acyl Transferase (ng/mL)						
Normal Weight	10.45±0.26	2.01±0.03	10.38±0.14	2.01±0.02	10.34±0.19	2.01±0.03
Obese	9.84±1.48	2.27±0.55	9.86±1.28	2.30±0.67	9.86±1.28	2.13±0.29

NW = normal weight; OB = obese; * indicates a significant pre-to-post change for the subgroup; Y indicates a significant difference between NW and OB. Note. All values reported are represented as means ± standard deviations, unless otherwise noted.

VO₂R ($z = -2.666, p = .008$) for 40 min and after the seated control session for 40 min ($z = -2.666, p = .008$); however, these findings were not different between adults with a NW and OB body weight status ($p > .05$).

Acylated Ghrelin

Means and standard deviations of acylated ghrelin for the total sample and by body weight classification are presented in Table 2. Mann Whitney U-tests revealed no significant differences in acylated ghrelin between NW and OB before and after performing 40 min of cycle exercise at 30% and 60% VO₂R ($p > .05$). Wilcoxon-signed rank tests assessed pre-to-post 40 minutes of cycling exercise at 30% VO₂R, 60% VO₂R, and in the control condition. A significant pre-and-post difference in acylated ghrelin was found for the total sample after 60% VO₂R for 40 min ($z = -2.429, p = .015$) but these findings were not different between NW and OB ($p > .05$).

Leptin

Means and standard deviations of leptin for the total sample and by body weight classification can be located in Table 2. Mann Whitney U-tests revealed significant differences in pre and post-exercise and control leptin between adult classified with a NW and OB body weight status before and after performing 40 min of cycle exercise at 30% and 60% VO₂R and after a 40 min seated control session ($p < .05$). Wilcoxon-signed rank tests assessed whether differences in leptin existed before and after the seated control session, 40 min of cycle exercise performed at 30% VO₂R and/or 40 min of cycle exercise performed at 60% VO₂R. No significant mean rank differences were found for the total sample after compared to before the seated control session, or after 40 min of exercise performed at 30% and 60% VO₂R ($p > .05$) and these findings were similar between adults classified with a NW and OB body weight status ($p > .05$).

Insulin

Means and standard deviations of leptin for the total sample and by body weight classification can be located in Table 2. Mann Whitney U-tests were performed to assess whether differences existed between adults with a NW and OB body weight status for pre and post insulin concentrations after a seated control session for 40 min, a cycle exercise session performed at 30% VO₂R for 40 min and a cycle exercise session performed at 60% VO₂R for 40 min. Significant differences in post-control compared to pre and post exercise insulin were found between adults with a NW and OB body weight status at 30% and 60% VO₂R. Wilcoxon-signed rank tests assessed whether differences in insulin existed before and after the seated control session, 40 min of cycle exercise performed at 30% VO₂R and/or 40 min of cycle exercise performed at 60% VO₂R. No significant mean rank differences were found for the total sample after compared to before the seated control session, or after 40 min of exercise performed at 30% and 60% VO₂R ($p > .05$) and these findings were similar between adults with a NW and OB body weight status ($p > .05$).

Perceived Hunger

Means and standard deviations for perceived hunger for the total sample and by body weight classification can be found in Table 3. Mann Whitney U-tests revealed no significant difference between NW and OB ($p > .05$). Wilcoxon-signed rank tests revealed no significant mean rank differences for the total sample after compared to before the seated control session, or after 40 min of exercise performed at 30% and 60% VO_2R ($p > .05$) and these findings were similar between NW and OB ($p > .05$).

DISCUSSION

The purpose of the current study was to examine the influence of acute AE on circulating appetite regulating hormones, GOAT and perceived hunger in adults classified with a NW and OB body weight status. The major findings from the study were that GOAT concentrations were similar between NW and OB across all conditions of the study protocol and acylated ghrelin decreased after compared to before 30% and 60% VO_2R . Leptin and insulin concentrations were also found to be statistically different between NW and OB before and after exercise and control but were not changed differentially pre-to-post exercise and in the control condition. Likewise, perceived hunger was similar between adults with a NW and OB body weight status across exercise and control conditions, respectively.

Synthesis of the Study Findings to Previous Literature

To date, several researchers have attempted to explore the influence of AE on circulating ghrelin, leptin, and insulin in adults with a NW body weight status (Bogardus, 1988; Bouassida, 2006; Broom, 2007; Essig, 2000; King, 2011; Olive, 2001; Perusse, 1997; Romjin, 1993; Schmidt, 2004; Weltman, 2000), few have attempted to explore the influence of exercise in adults with an overweight and OB body weight status (Colberg, 1996; Minuk, 1981; Tiryaki-Sonmez et al., 2013); and none have explored the influence of exercise on GOAT to the best of our knowledge. From the studies conducted, researchers have reported either no change or an acute post-exercise reduction in plasma and acylated ghrelin in men with a NW body weight status and women with an OB body weight status (Broom, 2007; King, 2011; Schmidt, 2004; Tiryaki-Sonmez et al., 2013), which are similar to our findings. Our observed changes in GOAT across conditions and acylated ghrelin pre-to-post-exercise performed at 30% and 60% VO_2R for 40 min indicate that exercise performed at light and vigorous intensities may be warranted when at-

tempting to suppress appetite and hunger as non-pharmacologic lifestyle therapies for the treatment and management of OB; however additional studies replicating these findings in larger samples of men and women with OB are encouraged. Previous studies exploring the acute and chronic effects of AE on plasma leptin and insulin demonstrated AE to confer little to no benefit on leptin or insulin secretion, after controlling for demographic and training induced changes in body composition (Bogardus, 1988; Borghouts, 2000; Bouassida, 2006; Colberg, 1996; Essig, 2000; Kraemer, 1999; Mikines, 1988; Olive, 2001; Perusse, 1997; Romjin, 1993; Torjam, 1999; Weltman, 2000; Young, 1989). Such findings are in accordance with the findings of the current study as we did not observe significant pre-to-postexercise and control differences between NW and OB; however, our pre-and post-exercise and post-control findings affirm higher plasma leptin and insulin in OB than NW, which have been well documented in the literature as functions of leptin and/or insulin signaling and leptin and/or insulin resistance (Borer, 2013; Considine, 1996).

Proposed Mechanisms for the Study Findings

A number of mechanisms may explain the significant pre-to-post exercise and control changes found in GOAT and acylated ghrelin for the current sample in our pilot study. First, sympathetic tone increases with exercise, promoting the redistribution of blood from the splanchnic region to the peripheral tissues, thereby delaying transport of acylated ghrelin to the growth hormone secretagogue receptor (GHS-R) in the arcuate nucleus of the ventromedial hypothalamus (King, 2011). Consequently, as GOAT is co-secreted with acylated ghrelin from the submucosa of the gastric fundus, increases in sympathetic tone may therefore delay transportation of this gut enzyme and hormone in the circulation. Second, adults with an OB body weight status have an attenuated suppression of ghrelin compared to those with a NW body weight status, causing delays in the secretion and/or binding of leptin to functional leptin receptors (LepRp) in the ventral lateral hypothalamus. (Klok, 2007) reviewed literature pertaining to the role of leptin and ghrelin on the regulation of energy balance among NW and OB and reported that leptin activation at the hypothalamus is attenuated in adults with an OB body weight status.

Proposed mechanisms explaining the purported attenuation in the activation of leptin in OB included alterations in Lep-Rp signaling and/or alterations in the manner in which leptin crosses the blood brain barrier. To support this hypothesis, (Caro, 1996) examined the ratio of cerebrospinal fluid

Table 3. Perceived hunger by body weight classification before and after exercise at 30% and 60% maximum oxygen uptake reserve and seated control

Perceived Hunger	30% VO_2R		60% VO_2R		Seated Control	
	Pre	Post	Pre	Post	Pre	Post
Normal Weight	9.50±1.00	10.25±3.10	10.50±2.08	10.00±2.45	9.25±3.59	10.75±4.27
Obese	6.40±3.29	8.40±2.61	9.60±1.67	9.80±3.63	8.40±4.10	9.80±2.49

All values reported are represented as means±standard deviations, unless otherwise noted.

leptin (CSF) to blood leptin in adults with a NW, overweight and OB body weight status ($n = 31$) and revealed that the ratio of leptin within CSF compared to serum was lower in OB compared to NW. The authors indicated that a higher proportion of triglycerides in OB may saturate the leptin transporter and therefore inhibit the quantity of leptin that can cross the blood brain barrier in adults with an OB body weight status. Notwithstanding, adults with an OB body weight status have approximately 30% more leptin in CSF and a similar number of Lep-RP compared to those with a NW body weight status, a Lep-RP deficit therefore provides a plausible explanation for the inability of adults with OB to adequately regulate appetite suppression at rest and in response to exercising situations. Lastly, to accompany the second mechanism of leptin resistance, an alteration in the signal transducer and activator of transcription 3 (STAT3) and phosphoinositide 3 kinase (PIK3) pathways in OB has been hypothesized. Oswal, 2010 reviewed the influence of central targets and signaling mechanisms on the pathogenesis of OB and reported that mutations in Lep-RP inhibit the expression of STAT3. The authors also reported that blockage to PIK3, an enzyme involved in cellular function, can inhibit the sensitivity of proopiomelanocortin (POMC) neurons in the ventrolateral hypothalamus to leptin, thereby inhibiting the suppression of hunger in OB, making the treatment and management of overweight and OB more challenging (Oswal, 2010).

Limitations and Strengths of the Study

The present study is not without limitation. Despite performing a preliminary power analysis to establish an effect size with 80% power, our sample in this pilot study remained small but adequately powered. Moreover, we assumed that all participants adhered to all pretesting instructions for all treatment and control sessions, and that all measurements taken from the baseline health fitness assessment yielded accurate and reliable information for the classification of participants to group membership and the prescription of exercise to a relative load of 30% and 60% VO_2R for two of the three randomized testing sessions. Other limitations of the study included an inability to control for the reactive influence of the participant to the treatment or control conditions administered.

The strengths of the current study lie in the research design. The use of a quasi-experimental design with predetermined and rigorous inclusion/exclusion criteria allowed our team to randomly assign and counterbalance the AE and control sessions to subjects classified as NW or OB. The use of a standard exercise intervention based upon current ACSM recommendations was a second strength of the current study as we were able to prescribe cycle exercise at 30% and 60% VO_2R for 40 min per session. Other strengths of the study included the detection (0.78-50 ng/mL), sensitivity (<0.27 ng/mL), precision (intra-assay CV $<10\%$; inter-assay CV $<12\%$), and stability ($<5\%$) of the biochemical assay, and the consideration of potential confounders of stress and sleep quality that might impact the influence of exercise on circulating concentrations of GOAT among NW and OB, respectively.

CONCLUSION

In summary, the purpose of the current study was to examine the influence of acute AE on circulating appetite-regulating hormones and GOAT in a pilot sample of adults with NW and OB body weight classifications. GOAT and acylated ghrelin were found to significantly decrease pre-to-postexercise and control between NW and OB, whereas leptin and insulin remained similar but differed between NW and OB at pre-and-post-exercise and control. Our findings demonstrate that AE performed at 60% VO_2R may be necessary to acutely suppress appetite-regulating hormones responsible for signaling hunger and appetite sensations; but, should be replicated and further substantiated in larger, more racially and ethnically diverse samples of men and women with OB. Our findings support the premise that leptin and insulin, while not significantly reduced pre-to-post exercise and control in the current study, may be functional targets in future exercise trials along with acylated ghrelin, GOAT and other gut peptide hormones seeking to modulate biological hunger and hedonic appetite in an interdependent fashion such that energy expenditure may influence energy intake.

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