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Clinical Study

Influence of Running and Walking on Hormonal Regulators of Appetite in Women

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Nine female runners and ten walkers completed a 60 min moderate-intensity (70% VO_2max) run or walk, or 60 min rest in counterbalanced order. Plasma concentrations of the orexogenic peptide ghrelin, anorexogenic peptides peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and appetite ratings were measured at 30 min interval for 120 min, followed by a free-choice meal. Both orexogenic and anorexogenic peptides were elevated after running, but no changes were observed after walking. Relative energy intake (adjusted for cost of exercise/rest) was negative in the meal following running (-194 ± 206 kcal) versus walking (41 ± 196 kcal) ($P = 0.015$), although both were suppressed ($P < 0.05$) compared to rest (299 ± 308 and 284 ± 121 kcal, resp.). The average rate of change in PYY and GLP-1 over time predicted appetite in runners, but only the change in GLP-1 predicted hunger ($P = 0.05$) in walkers. Results provide evidence that exercise-induced alterations in appetite are likely driven by complex changes in appetite-regulating hormones rather than change in a single gut peptide.

1. Introduction

The benefits of exercise in the prevention of chronic diseases including overweight and obesity are well documented. Regular physical activity reduces blood pressure, creates a more favorable lipid profile, and reduces risk for stroke, coronary heart disease, hypertension, and colon cancer [1, 2]. Regular exercise also helps maintain healthy body weight [1] and may aid in weight loss and weight loss maintenance [3]. To help prevent weight gain (or obesity), the 2008 Physical Activity Guidelines for Americans [4], sponsored by the Centers for Disease Control and Prevention and Healthy People 2020, suggests incorporating a minimum weekly total of two and a half hours of moderate-to-vigorous intensity physical activity, spread over most days of the week. Working up to five or more hours per week (~60 min/day) is recommended to gain additional benefits which include weight loss and weight loss maintenance.

Although the aforementioned recommendations, if followed, are likely to have a major impact on health, intervention studies find that exercise without intentional food restriction and/or behavior modification does not effectively promote weight loss, [5, 6], particularly in women [7, 8]. This may be because exercise stimulates a compensatory (relative to the energy expenditure of the activity) or noncompensatory drive to eat that is either biologically—(i.e., altered appetite regulating hormones) or psychologically—(i.e., feeling one deserves dessert after exercising) driven. These studies, however, are not consistent with short-term experimental studies conducted mostly in men which have found reductions in appetite and relative food intake following moderately intense-to-vigorous exercise. This may be because the exercise-induced effect is influenced by factors including the intensity and mode of the exercise [9–11], the sex, and body composition of the exerciser [9, 10]. Several previous studies found that hunger and/or food intake are

suppressed following 30–90 min of intense- but not necessarily light-to-moderate intensity exercise [12–18] including cycling, running, and brisk walking. Others reported increases in hunger and food intake following swimming [19] and exercise calisthenics [20]. Less is known concerning individual differences; however, one study found suppressed hunger and food intake in lean but not overweight women following bicycle exercise [21].

The recent discovery of several gut peptides involved in appetite regulation and energy homeostasis provides an attractive mechanism to explain how exercise reduces hunger/appetite in some conditions and increases it in others. Alterations in circulating ghrelin, the only known orexigenic gut peptide, along with the anorexigenic gut peptides peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) may work in concert to influence exercise-associated alterations in hunger and food intake. Alterations in circulating gut peptides appear to regulate food intake for as long as 24 h and are not specifically controlled by body fat stores. A number of previous studies have found that these peptides are altered by an acute bout of exercise [16, 17, 22–28]; however, the majority of studies evaluated only a single mode of exercise compared to rest. In addition, only a few of these studies simultaneously evaluated both the orexogenic and anorexogenic gut peptides [17, 27, 28], and few included women [23, 27, 28].

The purpose of this study was to assess the effect of a 60-minute bout of exercise on circulating concentrations of gut peptides ghrelin, PYY, and GLP-1; appetite and *ad libitum* food intake among women. An additional purpose was to assess whether alterations in these gut peptides were associated with alterations in appetite following exercise. Exercise was performed at a moderately hard intensity in two different modes: running and walking. We hypothesized that circulating ghrelin would be suppressed; PYY and GLP-1 concentrations elevated following both modes of exercise compared to rest. Furthermore, we hypothesized that ghrelin concentration would be directly correlated with ratings of hunger and desire to eat and PYY and GLP-1 concentrations would be indirectly correlated.

2. Methods

Nine endurance-trained female runners and ten habitual walkers between the ages of 18–40 were recruited for the study. To qualify, participants had to be in good general health, have normal hemoglobin (between 14.0–18.0 mg/dL) and thyroid status (thyroid stimulating hormone between 0.40–4.50 mlU/L), have regularly occurring menstrual cycles, and be of "low exercise risk" as per the American College of Sports Medicine (ACSM) [29]. The runners had to be currently running at least 32 km/wk, be performing runs of at least 60 min in duration as part of their training regiment, and have maximal aerobic capacity (VO_2max) of at least 45 mL/kg/min. The walkers had to be performing walks of at least 60 min in duration three or more days/wk and have a VO_2max of less than 40 mL/kg/min. Participants were excluded if they smoked, were anemic, hyper-or hypo-thyroid, pregnant or postmenopausal, had renal, hepatic, endocrine, gastrointestinal, pulmonary, cardiac, or hematological diseases including high blood pressure (>120/80 mm/Hg at rest), prediabetes/diabetes, demonstrated signs of significant depression, anxiety, other psychological problems, alcoholism or other substance abuse, used prescription or over the counter medications (other than contraceptives), or herbal preparations that can influence metabolism, had food allergies, or were unwilling to consume all foods/beverages provided in the run-in diet. The study was approved by the Institutional Review Board of the University of Wyoming. Volunteers were fully informed of possible risks of all procedures before providing written informed consent.

2.1. Baseline Testing. Approximately two weeks before initiation of the experimental protocol, VO_2max was determined on a motor-driven treadmill (Trackmaster TMX22, Newton, KS, USA) in accordance with ACSM recommendations [29]. For most runners, testing was initiated at 6 mph (0% grade) with the grade increasing by 1% every min until exhaustion. For the walkers, the test was initiated at 3.5 mph (2% grade) with grade increasing by 1% every min until exhaustion. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured continuously using a metabolic cart (ParvoMedics TureOne 2400, Sandy, UT, USA), and heart rate (HR) was monitored by an electrocardiography machine (Quinton Q-5000, Bothell, WA, USA). Rating of perceived exertion (RPE) was assessed during the last 10 seconds of each stage using the modified Borg Scale [29]. The highest 20-second VO_2 and respiratory exchange ratio (RER) achieved in the final two min of exercise were recorded as the maximum values. To qualify as an acceptable maximum test, participants had to meet two of the four following criteria: (1) a leveling or plateau of VO_2 (defined as an increase of $<2\,\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with increased workload); (2) RER $\geq$ 1.10; (3) maximum heart rate within 10 beats of age predicted maximum [$208 - (0.7 \times \text{age})$] [30]; (4) rating of perceived exertion (RPE) $\geq$ 17. After a 30 min recovery period, participants underwent a titration run/walk to determine the speed and grade required to elicit an oxygen uptake of 70% VO_2max. For descriptive purposes, body composition was measured using dual-energy X-ray absorptiometry (DEXA, GE Lunar Prodigy 8743, Waukesha, WI, USA).

2.2. Experimental Protocol. The study was a counterbalanced, cross-over study where participants completed an exercise and control (rest) test day. A schematic of the study is shown in Figure 1. The two test trials were scheduled in the follicular phase of the participants' menstrual cycle (between days 1 and 11) and spaced either 2 to 10 days or 1 menstrual cycle (3 to 5 wks) apart. The exercise test day consisted of a 60 min run/walk at 70% VO_2max followed by 2 h of rest, whereas the control day consisted of 3 h of rest. Food intake was controlled for 24 h prior to each test day by providing participants with a controlled diet. The diet provided 2000 kcal (64% carbohydrate, 14% protein, and 22% fat) from commercially available foods and beverages plus an optional additional 200 kcal provided as two 100 kcal snack bars (28.4 g, ~100 kcals; Clif Bar and Company, Berkeley, CA, USA).

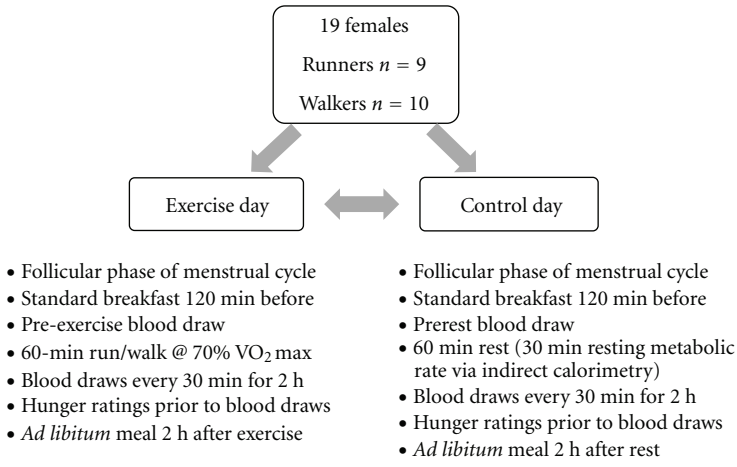

Figure 1: Schematic of the counter-balanced cross-over study.

Volunteers were asked to consume the foods provided (and nothing in addition other than water) and to return empty wrappers and any food and beverages that could not be consumed.

2.3. Test Days. On both test days, participants consumed a standard breakfast (Boost Smoothie, Clif Builder Bar and 2 cups of water; ~380 kcal; 65% carbohydrate, 20% protein, 15% fat) at 0630 prior to arriving in the laboratory. At 0730, height, weight, and blood pressure were measured. On the control visit, an intravenous indwelling (IV) catheter was inserted into an arm or hand vein and connected to a normal saline solution (0.9% saline solution) that was slowly infused to keep the catheter patent. Blood was drawn immediately before (baseline, preexercise) and immediately after one h of rest ($t = 0$ min) and every 30 min thereafter for 2 h ($t = 30, 60, 90, 120$ min). The first 3 cc at each time point draw was presumed to be diluted with saline and was discarded. Resting energy expenditure (REE) and respiratory quotient (RQ) were measured using a metabolic cart (160 lpm pneumatach, ParvoMedics, TrueOne 2400, Sandy, UT, USA) while the subject lay motionless in the supine position, as previously described [31]. The last 20 min of data was used to calculate REE.

On the exercise day, baseline blood was obtained by venipuncture. An IV catheter was inserted immediately following the 60 min run/walk, and blood was drawn on the same schedule as the rest day. For 10 min at the beginning (between 5–15 min after the start) and end (last 5 min) of the run/walk session, VO_2, VCO_2, RER and RPE, were measured using a metabolic cart (800 lpm pneumatach, ParvoMedics, TrueOne 2400, Sandy, UT, USA). Exercise pace was adjusted, if necessary, during the first 10 min to achieve an oxygen cost as close to 70% VO_2max as possible but was not further adjusted. HR was monitored continuously using a portable heart rate monitor (Polar S60i, Polar, Port Washington, NY, USA).

On both test days, hunger and satiety were assessed using 100 mm visual analogue scales (VASs), anchored at each end with a word describing the extremes of the appetite being measured [32]. The scales specifically asked (1) how hungry do you feel? (2) how satisfied do you feel?; (3) how full do you feel? (4) how much do you think you can eat? Hunger and satiety ratings were obtained 4 to 5 min before each blood draw ($t = 0, 30, 60, 90$, and 120) and at 20 min after initiation of the *ad libitum* meal (see below).

At completion of the exercise and rest sessions (120 min), participants were offered an *ad libitum,* free-choice meal. The free-choice meal consisted of the following (in weighed portions) attractively and consistently arranged on the dining table: rigatoni pasta (140 g dry, cooked), marinara sauce (140 g), alfredo sauce (140 g), whole-wheat bread (2 slices), white bread (2 slices), hard boiled eggs (2), apples (2), oranges (2), Clif snack bar (2), Clif Builder bar (2), nonfat yogurt (1, 6 oz), regular yogurt (1, 6 oz), individual portions of margarine (4), honey (4), peanut butter (4), assorted jellies (4), lemon-lime Gatorade (2, 20 oz), 2% milk (2, 8 oz), and water (~1500 g). Participants were given 20 min to eat the meal and were instructed to eat until satiety. Participants were not allowed to read or study during the meal or carry backpacks, purses, or coats into the room. They were discretely monitored by the same investigator who worked quietly on a computer in the back of the room (with their back turned toward the participant). Food and water consumption were determined by weighing remaining food (to the nearest 0.1 g) at cessation of eating. By difference, food/beverages consumed were analyzed for total energy, protein, fat, carbohydrate, simple sugars, and fiber using Nutritionist Pro (Axxya Systems, Stafford, TX, USA, 77477). *Ad libitum* water intake and intake of energy and macronutrients as solids and liquids were also assessed. Relative food intake was calculated by subtracting estimated energy expenditure during the exercise (60 min exercise, 120 min rest) or rest (180 min rest) sessions from the respective free-choice energy intake.

2.4. Blood Samples and Hormone Analysis. Blood samples taken both before exercise and rest (baseline) were analyzed for serum concentrations of progesterone concentration

using a human solid phase RIA kit (Siemens Diagnostics, Los Angeles, CA, USA). Plasma samples were analyzed for total ghrelin, acylated ghrelin ($ghrelin_{acyl}$), $PYY_{3\text{-}36}$, GLP-1, glucose, lactate and hematocrit at pre-exercise/rest and for 120 min following exercise and rest were analyzed for total ghrelin, acylated ghrelin ($ghrelin_{acyl}$), $PYY_{3\text{-}36}$, GLP-1, glucose, lactate, and hematocrit. Blood samples for total ghrelin, $ghrelin_{acyl}$, $PYY_{3\text{-}36}$, and GLP-1 were collected into EDTA-treated prechilled tubes. Blood collected for analysis of plasma $ghrelin_{acyl}$ was collected into a chilled tube containing 100 μL of 200 mM AEBSF with 200 μL of 1 N HCl added per mL of plasma following centrifugation. Samples collected for analysis of PYY were treated with 150 μL of aprotinin and 40 μL DPP-IV. All plasma samples were cold-centrifuged (2–8°C) for 10 min at 3500 rpm. Aliquots of supernatant were stored in cryovials at −80°C and batch-analyzed in duplicate at study completion by radioimmunoassay using commercially available kits specific for humans (Millipore, St. Charles, MO, USA). Blood samples for analysis of glucose and lactate were collected into 4 mL purple top vacutainers, centrifuged as above, and the plasma was stored at −80°C until analysis. Glucose and lactate were analyzed using a Microstat Multiassay Analyzer (Analox instruments, Lunenburg, MA, USA). Hematocrit was analyzed as a marker of hemoconcentration and hemodilution using an Autocrit Ultra 3 (Clay Adams, Sparks, MI, USA) at each blood draw.

2.5. Statistical Approach. A sample size analysis conducted with mean and standard deviation estimates based on preliminary exercise-associated data from Russel et al. [28] in women ($n = 10$) and Martins et al. [33] in both sexes ($n = 6$ men, 6 women) for the pre-to post-exercise change in $PYY_{3\text{-}36}$ (15 to 25% increase with exercise; SDs proportional to means; ratio of SD to mean = 0.26) and an alpha = 0.05 determined that a sample size of $n = 8$ was sufficient to detect, with 80% power, a minimal postexercise increase of ~20% (http://www.statsalive.com/). Given this calculation, an $n = 9$ for each exercise group (i.e., one additional subject per group) was selected. The sample size calculation was not performed using ghrelin or $ghrelin_{acyl}$ due to inconsistent results for ghrelin and lack of published results for $ghrelin_{acyl}$.

Our first aim was to assess the effect of exercise on circulating concentrations of gut peptides, appetite, and *ad libitum* food intake among runners and among walkers. Concentrations of the gut peptides (total ghrelin, $ghrelin_{acyl}$; $PYY_{3\text{-}36}$ and GLP-1), and the primary measure of appetite, hunger ratings were measured at baseline and five time points ($t = 0$, 30, 60, 90, and 120 minutes) following exercise and rest. As secondary outcomes relating to appetite, we also considered ratings of satiety, fullness, and desire to eat. For each subject, we summarized the repeated measures by calculating the slope or the rate of change in outcome per 30-minute interval, following exercise and rest. Using the derived slopes as the outcome, linear regression models were fit to evaluate differences between exercise and rest responses over the entire period of 120 minutes following exercise or rest. We adjusted for baseline levels and included an interaction between exercise/rest and runner/walker group. We calculated robust standard errors that accounted for correlation between exercise and rest measures from the same subject. As a way of capturing total response over all time points, the area under the curve (AUC) was also calculated for the 120 minutes following exercise and rest for the gut peptide concentrations and hunger ratings using the trapezoid method (GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego, CA, USA, http://www.graphpad.com/). AUC included, by definition, the area under the curve and above baseline. For ghrelin, $ghrelin_{acyl}$, and GLP-1 which were observed to dip below baseline in the later postexercise period, negative AUC was also calculated as the area above the curve and below baseline. Paired t-tests were used to evaluate differences between exercise and rest responses over the entire period of 120 minutes after exercise or rest, within runners and within walkers. *Ad libitum* food intake was measured at a single time point following the *ad libitum* meal, as absolute energy intake and relative energy intake. Again, paired t-tests were used to compare exercise versus rest within runners and within walkers. As secondary outcomes, we also considered more specific components of energy intake, using three macronutrients: protein, carbohydrate, and fat. As an exploratory analysis relating to this aim, we also assessed the immediate effects of exercise versus rest on gut peptide concentrations and appetite. That is, rather than considering the trajectory of each outcome across all time points from $t = 0$ to $t = 120$, we considered only the difference between the measurements at $t = 0$ and at baseline for exercise versus rest. With these differences as the outcomes, we fit linear regression models, including an interaction between exercise/rest and runner/walker group. Again, we calculated robust standard errors that accounted for correlation between exercise and rest measures from the same subject.

Our second aim was to investigate whether changes in ghrelin, $ghrelin_{acyl}$, PYY, and GLP-1 were associated with changes in hunger following exercise. For each gut peptide, we fit a linear regression model with the peptide concentration as the predictor of interest and hunger as the outcome. Additionally, we included an interaction between hormone level and runner/walker group and adjusted for baseline hunger rating and exercise/rest period. Again, we calculated robust standard errors to account for multiple measures from the same subject. The other appetite ratings were also assessed as secondary outcomes. Statistical analyses were performed using Stata (version 10) and R statistical software (version 2.10.1). All reported P values were two-sided, with statistical significance taken to be P value < 0.05. There was no adjustment for multiple testing.

3. Results

Nineteen volunteers (9 runners and 10 walkers) enrolled in the study. The majority of the runners regularly competed in local road races and two were NCAA division I collegiate runners. The walkers walked regularly either for fitness and weight control, or as cross-training for other activities. None of the walkers, however, were regular runners or joggers.

Table 1: Baseline preexercise characteristics for 9 female runners and 10 female walkers. Data are presented as mean ± SD. Body fat percentage by dual-energy X-ray absorptiometry; VO_2max: maximal oxygen uptake while running or walking on a motor driven treadmill.

(a) Anthropometric characteristics

	Runners	Walkers	P value
Age (yr)	23.7 ± 2.4	24.6 ± 6.9	0.70
Height (cm)	163.9 ± 4.2	164.6 ± 8.6	0.80
Mass (kg)	53.5 ± 3.1	60.0 ± 12.3	0.14
BMI ($kg \cdot m^{-1}$)	19.8 ± 1.0	22.1 ± 3.4	0.06
Body Fat (%)	23.0 ± 4.9	35.7 ± 5.2	<0.001
VO_2max ($mL \cdot kg^{-1} min^{-1}$)	49.7 ± 3.0	33.9 ± 3.7	<0.001

(b) Gut peptide concentrations and appetite ratings before exercise and rest trials

	Runners			Walkers		
	Exercise*	Rest*	P value†	Exercise*	Rest*	P value†
Ghrelin	144.7 ± 52.6	167.8 ± 37.0	0.33	126.8 ± 42.8	209.4 ± 127.8	0.09
$Ghrelin_{acyl}$	10.6 ± 8.6	25.4 ± 15.7	0.02	6.4 ± 2.8	15.2 ± 7.2	0.22
PYY	45.0 ± 8.9	43.6 ± 10.9	0.67	51.2 ± 16.6	45.9 ± 13.6	0.39
GLP-1	42.5 ± 19.5	47.6 ± 15.5	0.59	55.2 ± 9.6	50.9 ± 27.0	0.63
Hunger	8.9 ± 9.4	13.6 ± 16.4	0.34	7.5 ± 9.7	17.7 ± 28.3	0.23
Satiety	71.8 ± 23.2	77.7 ± 19.3	0.75	79.6 ± 20.2	66.0 ± 28.9	0.11
Fullness	64.6 ± 26.4	78.2 ± 15.6	0.29	84.2 ± 11.4	73.6 ± 26.7	0.20
Desire to Eat	26.6 ± 25.1	33.4 ± 30.1	0.51	18.4 ± 14.2	32.4 ± 27.8	0.04

* Mean ± SD.
† Based on paired t-test.

Data from blood samples are missing for several participants at one or more time points following the exercise or rest periods due to complications from obtaining blood via the indwelling catheter (i.e., occasional clotting inadequate venous return). Data for $ghrelin_{acyl}$ are missing for several time points due to undetectable readings by the RIA.

3.1. Baseline Characteristics. The characteristics of the 9 runners and 10 walkers are summarized in Table 1. Table 1(a) shows that the exercise groups were fairly comparable with respect to age and height. Not surprisingly, the runners were leaner than the walkers and had a higher VO_2max than the walkers.

Comparing exercise versus rest, Table 1(b) and Figure 3 show unexpected differences at baseline (preexercise or rest) in mean $Ghrelin_{acyl}$ only among runners, whereas mean ghrelin, PYY, and GLP-1 were similar in both groups before exercise and rest periods. Differences in mean appetite ratings were also observed prior to exercise versus rest periods among both runners and walkers.

The energy and macronutrient intakes of the controlled diet were similar ($P > 0.05$) before exercise and rest in both the runner and walker groups. Runners averaged 1868 ± 380 kcal (14.1 ± 1.7% protein, 64.8 ± 3.2% carbohydrate, and 21.4±2.3% fat) before exercise and 2035±239 kcal (14±0.6% protein, 63.6 ± 1.3% carbohydrate, and 22.4 ± 1.1% fat) before rest. Walkers averaged 1770 ± 400 kcal (14.7 ± 1.5% protein, 63.8 ± 2.7% carbohydrate, and 21.5 ± 2.6% fat) before exercise and 1811 ± 357 kcal (14.9 ± 1.8% protein, 62.3 ± 1.8% carbohydrate, and 22.7 ± 1.5% fat) before rest. In the runners, the exercise test day fell on menstrual cycle day 5.9 ± 3.1, whereas the rest day fell on day 5.2 ± 2.9. In the walkers, exercise and rest days fell on 5.4 ± 3.4 and 4.4 ± 1.5, respectively. Serum progesterone concentrations were less than 2 ng/mL for all participants during both exercise and rest and averaged 0.66 ± 0.2 and 0.64 ± 0.19 for runners and 0.63 ± 0.33 and 0.46 ± 0.3 for walkers during exercise and rest, respectively, confirming follicular status.

3.2. Oxygen Uptake, Heart Rate and Energy Expenditure. Absolute VO_2, HR, RER, RQ, RPE, and energy expenditure (EE) during the 60 min bout of running or walking and rest along with concentrations of lactate and glucose are shown in Table 2. Runners ran at an average pace of 2.9 ± 0.18 m/s (6.5 MPH), and walkers walked at an average of 1.69 ± 0.1 m/s (3.77 MPH). Absolute VO_2 was higher during exercise in runners versus walkers, but relative VO_2 was similar ($P = 0.624$) and averaged 70.4 ± 4.1% and 68.6 ± 6.4% VO_2max during the run and walk, respectively. HR was also ~10 bpm higher during exercise in the runners compared to the walkers (172 bpm versus 162 bpm) but was similar between the rest trials. Energy expenditure was approximately 180 kcals higher during exercise in runners compared to walkers (483.1 kcal/h versus 305.1 kcal/h) but was similar at rest. Blood lactate concentration was also similarly elevated following the run and the walk.

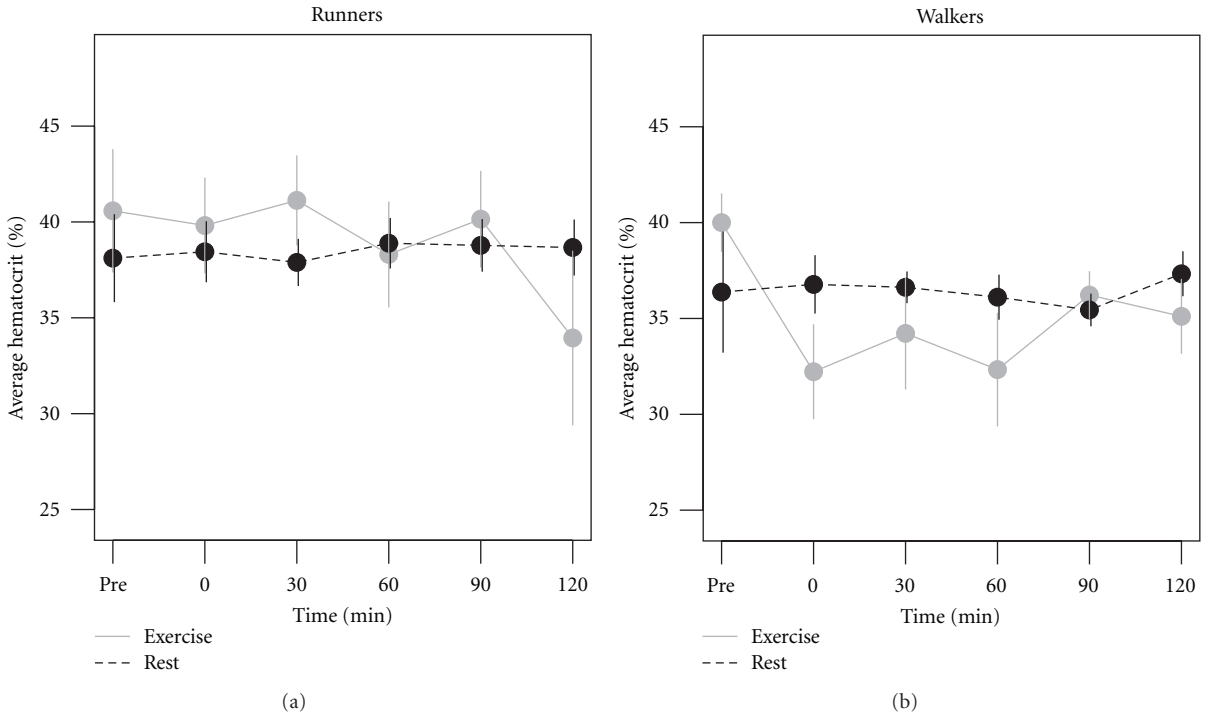

Figure 2: Average hematocrit for runners and walkers during exercise (solid grey) and rest (dashed black). "Pre" represents the time point just before exercise or rest. Vertical bars display the standard error of the mean hematocrit concentration at that time point.

Table 2: Oxygen uptake, energy expenditure, and heart rate of 9 female runners and 10 female walkers during 60 min of exercise and 60 min of bed rest. VO_2: volume of oxygen uptake; RER: respiratory exchange ration; RQ: respiratory quotient; HR: heart rate; RPE: rating of perceived exertion.

	Rest		Exercise			
	Runners*	Walkers*	Runners*	Walkers*	$\text{Runners}_{\text{Ex}}$ versus $\text{walkers}_{\text{Ex}}$†	P value
VO_2 ($L \cdot min^{-1}$)	0.23 ± 0.02	0.22 ± 0.04	1.9 ± 0.16	1.39 ± 0.34	0.48 (0.22, 0.73)	0.001
RER/RQ	0.78 ± 0.05	0.74 ± 0.05	0.90 ± 0.03	0.88 ± 0.04	−0.01 (−0.04, 0.02)	0.570
Energy expenditure (kcal/h)	65.5 ± 6.7	62.3 ± 11.5	483.1 ± 49.7	324.6 ± 138.1	158.5 (56.2, 260.7)	0.01
HR (bpm)	68.6 ± 24.3	72.3 ± 7.9	171.8 ± 11.3	161.9 ± 16.1	9.9 (−4.17, 24.0)	0.15
RPE	—	—	13.1 ± 1.1	14.1 ± 2.7	−0.97 (−3.13, 1.19)	0.35
Lactate (mmol/L)	0.76 ± 0.26	0.92 ± 0.4	1.4 ± 0.66	1.0 ± 0.55	0.4 (−0.21, 1.0)	0.19
Glucose (mmol/L)	5.1 ± 0.56	5.0 ± 0.67	6.7 ± 1.8	5.7 ± 0.66	1.03 (−0.38, 2.4)	0.14

* Mean ± SD.
† Difference in means (95% confidence interval) from two-sample t-test.
VO2: oxygen uptake; RER: respiratory exchange ratio; RQ: respiratory quotient; HR: heart rate; RPE: rate of perceived exertion.

3.3. Blood Concentration, Hormones, and Metabolites

Blood Concentration. As shown in Figure 2, total hematocrit was fairly constant over time following both exercise and rest in the runners and walkers. However, because hemoconcentration was observed following exercise in quite a few of the walkers and an occasional dilute samples from saline infusion was observed in both groups, blood data were adjusted according to the methods of Dill and Costill [34] and used in all statistical analyses. As a sensitivity analysis, we repeated all analyses using the unadjusted data and obtained similar results (data not shown).

Ghrelin. Figure 3 (upper left panel) illustrates the average trajectory over all time points (one time point before exercise or rest and five time point after exercise or rest). Average total ghrelin concentration drifted upward immediately after exercise in the runners and the walkers, and then leveled off in the runners, while demonstrating large variability in the walkers. The concentration remained fairly constant after rest. Table 3(a) reflects these results, indicating that exercise may increase the overall rate of change of ghrelin concentration in runners. While the rate of change after exercise stay close to zero (−2.1 pmol/L per minute), a positive average

Table 3: Effect of exercise on (a) rate of change of gut peptide concentrations and hunger ratings over 120 minutes after exercise or after rest, among runners and among walkers and (b) gut pepetide concentrations and hunger ratings immediately after exercise or after rest (at $t = 0$).

(a) Effect of exercise over all time points

	Runners				Walkers			
	Exercise*	Rest*	Exercise effect†	*P* value	Exercise*	Rest*	Exercise effect†	*P* value
Ghrelin (pmol/L)	10.0 ± 22.9	−2.1 ± 11.7	12.9 (−3.9, 29.7)	0.12	−12.8 ± 33.6	−4.6 ± 23.2	−9.9 (−42.6, 22.8)	0.53
$\text{Ghrelin}_{\text{acyl}}$ (pmol/L)	3.6 ± 10.0	−1.4 ± 3.9	2.9 (−7.6, 13.4)	0.56	1.7 ± 4.2	−1.3 ± 4.7	1.7 (−2.9, 6.3)	0.44
PYY (pmol/L)	−3.9 ± 2.4	−1.7 ± 1.6	−2.0 (−4.0, −0.095)	0.041	−9.4 ± 8.3	−2.2 ± 1.7	−6.7 (−13.2, −0.14)	0.05
GLP-1 (pmol/L)	−8.5 ± 7.9	1.5 ± 6.3	−10.7 (−17.0, −4.4)	0.002	−10.2 ± 10.6	6.9 ± 18.1	−16.5 (−28.0, −5.0)	0.01
Hunger	13.4 ± 5.9	10 ± 4.6	3.6 (−1.5, 8.7)	0.15	9.2 ± 4.9	7.6 ± 4.5	0.60 (−2.9, 4.1)	0.72
Satiety	−8.7 ± 7.8	−8.2 ± 7.6	−2.6 (−7.1, 1.8)	0.23	−5.6 ± 6.8	−7.6 ± 3.8	3.2 (−1.6, 8.0)	0.18
Fullness	−10.6 ± 6.4	−9.3 ± 3.3	−3.9 (−7.7, −0.16)	0.042	−8.2 ± 4.3	−6.6 ± 5.0	−0.35 (−3.9, 3.2)	0.84
Desire to Eat	10.2 ± 6.3	8.4 ± 4.3	1.9 (−2.4, 6.1)	0.37	7.9 ± 4.4	8.3 ± 3.7	−1.9 (−4.4, 0.67)	0.14

* Mean ± SD of slopes.
† Estimated effect (95% confidence interval) from linear regression, adjusting for baseline.

(b) Immediate effect of exercise

	Runners				Walkers			
	Exercise*	Rest*	Exercise effect†	*P* value	Exercise*	Rest*	Exercise effect†	*P* value
Ghrelin (pmol/L)	−3.7 ± 71.1	3.6 ± 43.5	−7.3 (−57.2, 42.6)	0.76	39.2 ± 125.4	−28.7 ± 151.9	68.0 (−70.0, 205.9)	0.31
$\text{Ghrelin}_{\text{acyl}}$ (pmol/L)	7.9 ± 5.7	0.03 ± 9.2	7.9 (−0.9, 16.7)	0.075	−2.3 ± 5.3	4.0 ± 20.5	−6.3 (−23.0, 10.3)	0.43
PYY (pmol/L)	8.3 ± 12.7	−4.0 ± 5.8	12.3 (3.6, 21.0)	0.008	9.8 ± 18.4	−3.6 ± 13.2	13.3 (1.9, 24.7)	0.02
GLP-1 (pmol/L)	30.3 ± 25.5	−2.2 ± 18.9	32.4 (5.3, 59.5)	0.022	9.8 ± 36.5	1.1 ± 10.5	8.7 (−23.8, 41.2)	0.58

* Mean ± SD of difference in t_0 and baseline.
† Estimated effect (95% confidence interval) from linear regression.

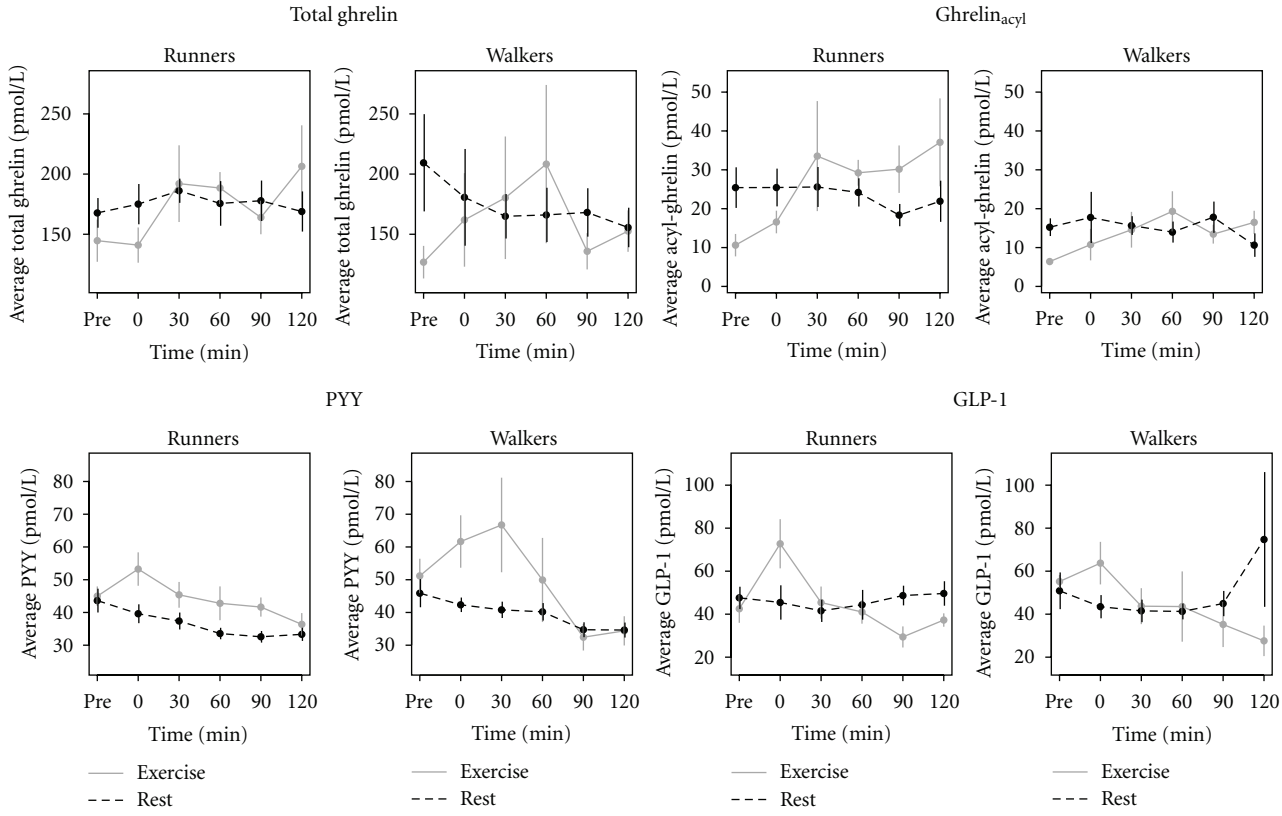

Figure 3: Average change in ghrelin (pmol/L, upper left panel), ghrelin$_{acyl}$ (pmol/L, upper right panel), PYY (pmol/L, lower left panel), and GLP-1 (pmol/L, lower right panel) for runners and walkers during exercise (solid grey) and rest (dashed black). "Pre" represents the time point just before exercise or rest. Vertical bars display the standard error of the mean concentration of gut peptide at that time point.

postexercise slope (10.0 pmol/L per 30 minutes) and an overall positive difference between exercise and rest (12.9 pmol/L per minute, 95% CI [−3.9, 29.7], P value = 0.12) are estimated. Meanwhile, for walkers, the larger variability at later time points may obscure the true effect of exercise. Table 3(b) contains results of exploratory analysis relating to immediate effects of exercise. Figure 3 (upper right panel) shows patterns for average Ghrelin$_{acyl}$ concentration that are somewhat similar to those for total ghrelin. Table 3(a) shows little evidence of an exercise effect on the rate of change of Ghrelin$_{acyl}$ concentration over time. There is some indication, however, of a larger immediate increase in Ghrelin$_{acyl}$ concentration after exercise versus after rest among runners (7.9 pmol/L, 95% CI [−0.9, 16.7], P value = 0.075).

The positive AUC (area above baseline) for both total ghrelin and ghrelin$_{acyl}$ was found to be higher following exercise in the runners but not the walkers. Negative AUC (area under baseline) was found to be smaller following exercise. Together, these results indicated that total response as measured by total ghrelin and ghrelin$_{acyl}$ tended to be higher after exercise than after rest among runners (Table 4).

PYY and GLP-1. In runners, PYY concentration peaked immediately after exercise (Figure 3) then gradually returned to baseline over the 120 min after exercise; whereas in walkers, PYY concentration peaked at 30 min after exercise before returning to baseline 90 min after exercise. Table 3(a) shows evidence of the effect of exercise on the rate of change of PYY, with exercise causing a faster decline in PYY concentration over time among runners (−2.0 pmol/L per 30 minutes, 95% CI [−4.0, −0.095], P value = 0.041) and among walkers (−6.7 pmol/L per 30 minutes, 95% CI [−13.2, −0.14], P value = 0.43), although the effect was not statistically significant in the walkers potentially due to high variability. Immediate effects were also evident, but positive (Table 3(b)), thus reflecting the observed pattern of an immediate rise in concentration followed by a decline among both runners and walkers. The positive AUC for PYY tended to be higher after exercise versus rest in the runners. Negative AUC was also found to be higher after exercise versus rest in walkers (Table 4).

Similar to PYY, GLP-1 concentration peaked immediately after exercise in both runners and walkers returning to pre-exercise concentrations at approximately 30 min after exercise. Unlike PYY, GLP-1 dipped visibly below baseline after 60 min after exercise in both groups (Figure 3). Table 3(a) shows fairly large effects of exercise on the rate of change of GLP-1. Again, exercise caused a faster decline in GLP-1 concentration among both runners (−10.7 pmol/L per 30 minutes, 95% CI [−17.0, −4.4], P value = 0.002) and walkers (−16.5 pmol/L per 30 minutes, 95% CI [−28.0, −5.0], P value = 0.008). There was evidence of an immediate effect of exercise among runners, but not in walkers. The positive AUC for GLP-1 was not different following exercise compared to rest in either the runners or the walkers, however,

Table 4: Effect of exercise on AUC values for hormones and metabolites. Results for both positive and negative AUC are presented.

Hormone/	Runners				Walkers			
metabolite	Exercise*	Rest*	Exercise effect†	*P* value	Exercise*	Rest*	Exercise effect†	*P* value
Ghrelin								
(+) area	20641 ± 16238	6322 ± 6450	14319 (340, 28298)	0.05	18157 ± 26247	6863 ± 9390	11294 (−10486, 33075)	0.27
(−) area	5672 ± 16471	3908 ± 7977	1764 (−12808, 16336)	0.79	6839 ± 8708	24574 ± 32687	−17734 (−44816, 9348)	0.17
$Ghrelin_{acyl}$								
(+) area	6246 ± 4933	700 ± 584	5546 (1822, 9271)	0.01	2067 ± 2437	1345 ± 1575	723 (−833, 2279)	0.32
(−) area	105 ± 183	1688 ± 2184	−1583 (−3172, 6)	0.05	813 ± 1671	946 ± 1283	−133 (−1297, 1031)	0.80
PYY								
(+) area	1796 ± 2667	59 ± 92	1737 (−290, 3764)	0.08	90 ± 163	1073 ± 2325	−983 (−2686, 719)	0.22
(−) area	2575 ± 2306	4211 ± 3103	−1635 (−4678, 1407)	0.25	6912 ± 6575	2415 ± 1483	4497 (−502, 9496)	0.07
GLP-1								
(+) area	1114 ± 926	693 ± 691	421 (−313, 1155)	0.22	556 ± 650	656 ± 618	−181 (−851, 488)	0.54
(−) area	802 ± 789	941 ± 1154	−139 (−1273, 995)	0.78	1957 ± 2058	1447 ± 3459	1658 (4, 3311)	0.05

* Mean ± SD of AUC.
† Difference in means (95% confidence interval) from paired *t*-test.

Table 5: Effect of exercise on absolute energy, relative energy, and macronutrient intake, among runners and among walkers.

	Runners				Walkers			
	Exercise*	Rest*	Exercise effect†	*P* value	Exercise*	Rest*	Exercise effect†	*P* value
Absolute energy intake (kcals)	485.8 ± 183.4	480.4 ± 126.4	5.5 (−112.0, 122.9)	0.917	623.9 ± 139.1	550.6 ± 162.4	73.2 (−11.0, 157.5)	0.08
Relative energy intake (kcals)	−193.9 ± 205.8	283.8 ± 120.6	−477.7 (−610.1, −345.3)	<0.001	126.8 ± 195.8	366.0 ± 183.6	−274.6 (−385.0, −164.3)	<0.001
Protein (g)	19.3 ± 6.0	19.3 ± 4.7	−0.046 (−4.1, 4.1)	0.980	27.6 ± 7.3	24.2 ± 9.5	3.4 (0.043, 6.8)	0.05
Carbohydrate (g)	80.5 ± 37.4	79.0 ± 20.9	1.5 (−20.6, 23.7)	0.877	88.6 ± 28.5	83.1 ± 22.2	5.5 (−13.0, 24.0)	0.51
Fat (g)	11.0 ± 3.5	11.3 ± 5.4	−0.38 (−4.3, 3.6)	0.830	19.2 ± 5.8	15.1 ± 7.4	4.1 (2.2, 6.0)	0.001

* Mean ± SD.
† Difference in means (95% confidence interval) from paired *t*-test.

the area below baseline was greater in the walkers (1957 ± 2058 and 1447 ± 3459, $P = 0.05$) but not the runners after exercise versus rest (Table 4).

3.4. Hunger and Satiety Ratings. As shown in Figure 4 (top panel), ratings of "hunger" increased from baseline across the 120 min after exercise or after rest period in both the runners and walkers during the exercise and rest trials. Similar results were observed for desire to eat, that is, how much you think you can eat (data not shown). Ratings of satiety (Figure 4, bottom panel) and fullness (data not shown) tended to decrease from baseline across the 120 min after exercise or after rest period in both groups during both trials. We see from Table 5 that there was not enough evidence to show an effect of exercise on the rate of change over time for any of the four subjective appetite ratings in either group. The AUCs for the four appetite ratings were also not found to be significantly different after exercise versus after rest for either group.

3.5. Ad Libitum Food Intake. As shown in Table 5, there was no evidence of a difference between absolute energy intake and macronutrient intake at the free-choice meal following running versus rest. However, absolute intake tended to be higher following walking compared to rest (73.2 kcals, 95% CI [−11.0, 157.5], P value = 0.080). Interestingly, walkers (but not runners) tended to consume more protein (in walkers: 3.4 g, 95% CI [0.043, 6.8], P value = 0.048) and fat (in walkers: 4.1 g, 95% CI [2.2, 6.0], P value = 0.001) following exercise compared to rest (Figure 5).

3.6. Relative Energy Intake. After adjusting for the cost of exercise or rest, relative energy intake was lower following exercise compared to rest in both groups (P 0.001). In

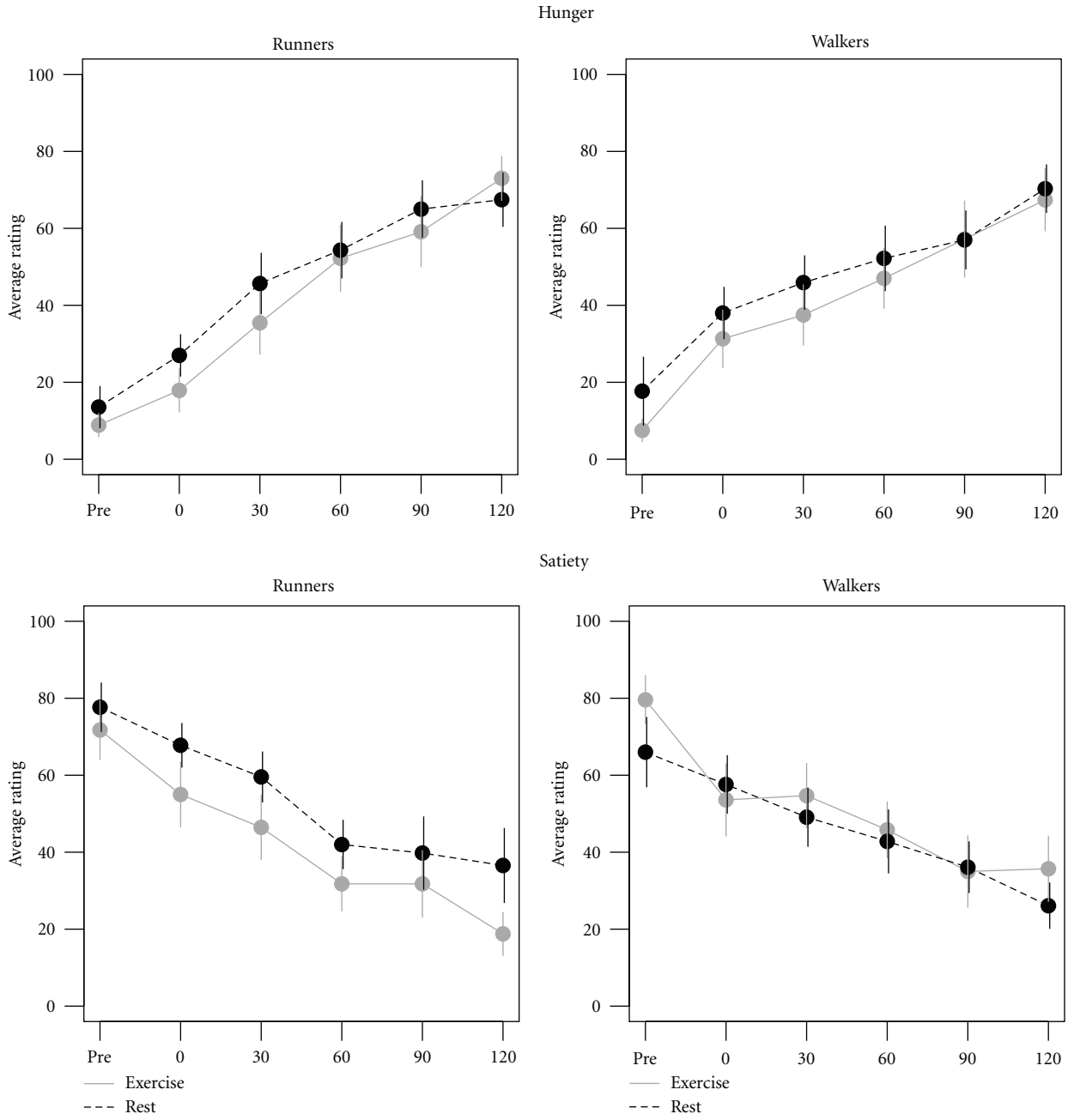

Figure 4: Average change in hunger (upper panel) and satiety (lower panel) for runners and walkers during exercise (solid grey) and rest (dashed black). "Pre" represents the time point just before exercise or rest. Vertical bars display the standard error of the mean for hunger and satiety at that time point. Data for fullness and desire to eat are not shown.

runners, relative energy intake was 477.7 kcals lower (95% CI [−610.1, −345.3]) after exercise compared to after rest. The difference was smaller in walkers, with after exercise being 274.6 kcals lower (95% CI [−385.0, −164.3]) than after rest.

3.7. Gut Peptides and Appetite Ratings. In the runners, the change in concentrations of PYY and GLP-1 was predictive of the change in hunger (Table 6). Analogous results were obtained for satiety, fullness, and desire to eat (data not shown). Increases in PYY and GLP-1 were positively associated with satiety and fullness and negatively associated with hunger and desire to eat. In the walkers, there may be some indication of an association between hunger ratings and $\text{Ghrelin}_{\text{acyl}}$ (0.63 units, 95% CI [−0.19, 1.46], P value = 0.12) and GLP-1 (0.095 units, 95% CI [−0.32, 0.028], P value = 0.095) concentrations.

4. Discussion

The purpose of the current study was to evaluate the effect of 60 min of moderately hard running and walking at the same

Table 6: Association of hormone concentrations with hunger ratings.

Hormone (pmol/L)	Hunger			
	Runners		Walkers	
	Hormone effect*	*P* value	Hormone effect*	*P* value
Ghrelin	0.038 (−0.066, 0.14)	0.45	0.033 (−0.022, 0.088)	0.22
$Ghrelin_{acyl}$	0.077 (−0.23, 0.38)	0.60	0.63 (−0.19, 1.46)	0.12
PYY	−1.02 (−1.52, −0.53)	<0.001	−0.034 (−0.28, 0.21)	0.78
GLP-1	−0.36 (−0.62, −0.11)	0.008	−0.14 (−0.32, 0.028)	0.09

* Estimate (95% confidence interval) from linear regression, adjusting for exercise and baseline hunger.

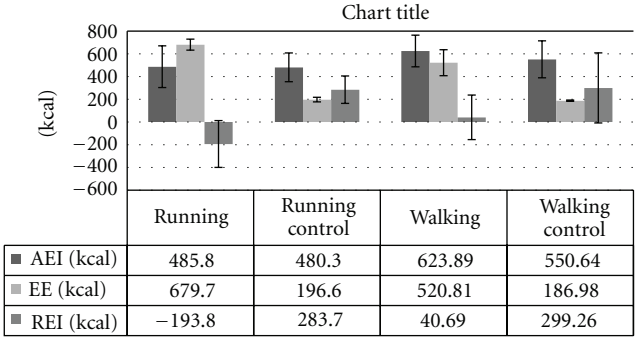

Figure 5: Absolute energy intake (AEI), relative energy intake (REI), and energy expenditure (EE) for exercise and rest in runners and walkers.

relative intensity (i.e., 70% of maximal oxygen uptake) on gut peptide concentrations, appetite, and food intake at a single *ad libitum* meal offered 2 h after exercise in habitually active women. Short-lived increases in circulating concentrations of the anorexogenic peptides and a trend for an increase in $ghrelin_{acyl}$ following exercise were apparent in the runners but not the walkers. These alterations in circulating gut peptides were associated with lower relative energy intake after exercise compared to rest which created a negative energy deficit in the runners but not walkers. Interestingly, the average rate of change in the anorexogenic peptides PYY and GLP-1 but not the orexogenic peptide ghrelin over time was found to predict hunger in runners but not walkers.

Ghrelin is secreted by specialized cells in the stomach and is currently the only known orexogenic peptide [35, 36]. Circulating concentrations of ghrelin peak during fasting, drop after a meal and are thought to be involved in hunger and meal initiation [37]. Peripheral infusion of ghrelin increases food intake in animals [38] and humans [39] through interaction with neuropeptide Y (NPY) and agouti-related protein (AgRP)-expressing neurons of the hypothalamic arcuate nucleus (ARC) and/or inhibition of vagal-afferent nerves [40]. Although ghrelin is present in circulation in acylated ($ghrelin_{acyl}$) and desacyl forms, only $ghrelin_{acyl}$ is thought to cross the blood-brain barrier and exert orexigenic effects [35, 40]. Thus, measurement of $ghrelin_{acyl}$, which accounts for ~10% of circulating concentration, is important particularly because $ghrelin_{acyl}$ responds more rapidly to glucose infusion [41] and exercise [42].

Our finding that the total response (i.e., AUC) of both total ghrelin and $ghrelin_{acyl}$ was elevated above rest following running but not walking at the same relative intensity is intriguing. Previous studies have observed decreases [17, 22, 26, 43, 44], increases [24, 45] or no alteration [18, 23, 27, 46] in both total ghrelin and $ghrelin_{acyl}$ concentrations following exercise, but these inconsistent findings may be due to the intensity (or energy cost) of the exercise employed and/or the sex of the exerciser. For example, total ghrelin was not altered by 60 min of submaximal cycling [27] but was increased following 3 h of prolonged cycling [24] and ~2 to 2.5 h of intense running [28]. $Ghrelin_{acyl}$ was also found to increase at a meal following treadmill walking in overweight women but not men [11] but only when the energy lost through exercise was not replaced. Overall, these results suggest that the energy cost of the exercise (which was ~38% higher during running versus walking) may promote increased ghrelin secretion, perhaps more so in women. Coupled with our finding that neither total ghrelin or $ghrelin_{acyl}$ correlated with hunger, the results also suggests that ghrelin is not a large contributor to postexercise food intake perhaps because the signal is dampened by increases in the anorexogenic peptides over the same time point [28].

In contrast to ghrelin, peptide YY and GLP-1 are satiety peptides which are secreted from the endocrine L cells of the distal gastrointestinal tract in response to a mixed meal [47]. Circulating concentrations of both PYY and GLP-1 are low in fasting and increase following meal ingestion [48]. Peripheral infusion of both peptides at physiological concentrations markedly decrease food intake in humans [49, 50] which appears to be additive when infused simultaneously [51]. The action of PYY is thought to be via inhibition of NPY/AgRP neurons and/or stimulation of vagal-afferent nerves, whereas the action of GLP-1 is thought to be via vagal mediation [37]. Both forms of PYY ($PYY_{3\text{-}36}$ and $PYY_{1\text{-}36}$) and GLP-1 are thought to serve as satiety signals, regulating the termination of individual meals [40].

Consistent with our findings, previous studies have found elevations in both PYY [25, 27, 52] and GLP-1 [27, 52] following different modes and intensities of exercise. A study by Ueda and colleagues [16] found that postexercise elevation of PYY but not GLP-1 was dependent on exercise intensity and was elevated to a greater extent following 30 min of cycling at 75% compared to 50% VO_2max. In another study, Broom and colleagues [17] found elevated PYY and suppressed hunger in the 2 h after a 60 min bout of

running at 69% VO_2max compared to both rest and a 90 min bout of resistance exercise. It is important to note, however, that the energy cost was 50% higher in the high- compared to the moderate intense cycling and ~260% greater with the running compared to the resistant training in the aforementioned studies. Thus, our finding that both PYY and GLP-1 were elevated immediately after running, and that only PYY was elevated after walking may also be explained by the greater energy cost of the run, which was ~37% greater than the walk. Interestingly, the average rate of change in PYY and GLP-1 after the run, and the rate of change in GLP-1 after the walk was significantly greater relative to rest, indicating an average downward trend following exercise, particularly for GLP-1 which dipped below baseline in the later postexercise period. While it is possible this dip in GLP-1, which had a more negative AUC in walkers compared to runners, at least partially accounted for the higher (less negative) relative intake in the walkers compared to the runners, future research is needed to affirm that such a role is causal.

Our results concerning *ad libitum* food intake following exercise in women are in agreement with previous studies in both sexes which found either no difference or slightly higher absolute food intake after a bout of exercise compared to a noexercise control, but significantly lower relative energy intake when accounting for the energy cost of exercise [12, 13, 53–55]. Interestingly, in these studies, relative energy intake was lowest (i.e., creating a more negative balance) when exercise intensity was high, and when foods offered in the subsequent *ad libitum* meal were low in fat [13, 53, 54]. Imbeault and colleagues [15], for example, found lower relative energy intake after 34 min of running at 75% VO_2max than after 72 min of walking at 35% VO_2max, which elicited the same energy cost (~485 kcal). King and colleagues [12], who were first to introduce the concept of relative energy intake, have argued the greater relevance of relative rather than absolute energy intake because higher energy intake would be an expected compensatory mechanism of increased energy expenditure through increased physical activity. Thus, if energy intake remains the same following exercise, as in the current study, it can be considered equivalent to a suppression of appetite relative to the intake expected to compensate for the exercise. Unfortunately, the majority of studies, including the current study, have not measured energy intake for a long enough period after exercise to evaluate how compensation for negative energy balance occurs following different modes of exercise like running but not necessarily walking. Total or partial compensation through altered energy intake and reduced energy expenditure are possible and likely, otherwise exercise would result in drastic reductions in body mass/body adiposity.

Although we did not find significant differences in perceived hunger at any point following running or walking compared to rest, small changes in hunger due to exercise rather than time (observed in the nonexercise control condition) may be difficult to detect using available methodology. Indeed, only about half of the studies in men using designs similar to ours have observed differences in hunger using VAS [12, 14, 16, 17, 22, 23, 27, 53, 56], whereas very few studies in women have detected exercise-associated differences [55, 57]. The lack of a strong exercise influence on appetite in all studies may be because VAS are not sensitive enough to detect small changes following exercise using sample sizes typically employed for exercise studies. It also may be that only a small subset of subjects is in tune with biological hunger cues and respond instead to other signals including time of day or time past since the last meal. Mattes [58], for example, observed that food intake often occurred when hunger was low or had not changed acutely. In our studies we did find, however, that VAS track well with changes in both PYY and GLP-1 in runners and tended to track with GLP-1 in walkers which suggests a relation between appetite ratings and satiety peptides even if exercise-induced alterations in appetite were not observed.

The current study used a unique complex modeling approach to evaluate whether changes in the gut peptides tracked with or predict changes in hunger and/or *ad libitum* food intake. Collectively, our findings suggest that changes in PYY and GLP-1 over time tracked indirectly with changes in hunger and desire to eat, and directly with changes in satiety. Interestingly, the change in either total ghrelin or $ghrelin_{acyl}$ did not track with subjective ratings of hunger. This provides additional support for the hypothesis that signals from elevated concentrations of circulating ghrelin may be muted by elevated concentrations of satiety peptides. Given that few [16, 22, 25] previous studies have found clear associations between gut peptide concentrations and appetite following exercise, it is probable that exercise-induced alterations in appetite are driven by complex changes in appetite-regulating hormones rather than a single gut peptide in isolation. A previous study by Martins and colleagues [27], for example, observed an inverse temporal pattern between hunger and both PYY and GLP-1 concentrations during 1 h of exercise but did not describe such a relation following exercise. In contrast, Broom et al. [22] and Ueda et al. [16] observed direct associations between the AUC for plasma $ghrelin_{acyl}$ and hunger, and indirect association between the AUC for GLP and postexercise energy intake. The discrepancy between the findings and published studies may be explained by the different exercise-induced patterns of gut peptide release.

In the current study, we elected to evaluate the effect of walking and running on appetite and gut hormone responses because both weight-bearing activities are recommended for weight loss and weight loss maintenance. Walking, however, is the most common exercise recommended [18] and, unlike running, can be undertaken by the majority of the population because it does not require the fitness base or produce the biomechanical stress of running. Our overall observation that walking did not elicit the same negative energy balance or increase in the satiety hormones as did running, yet promoted a slightly higher postexercise fat and protein intake, suggests that walking may create some challenges for long-term weight loss unless dietary restriction is employed. While our results appear to contradict those of King and Colleagues [18] who observed significantly lower relative energy intakes in men after a 60-min "brisk" walk at a self-selected pace (ranging from 33.8 to 55.5% VO_2max), the apparently discrepant results may help explain why exercise is less effective in promoting weight loss in women compared to men [7, 8].

The mechanism, however, may not be easily identified because $Ghrelin_{acyl}$ was not altered by walking in either study, and King and colleagues [18] unfortunately did not simultaneously measure PYY, GLP or other satiety peptides. In our study, we also observed a curious tendency for $Ghrelin_{acyl}$, total ghrelin and subjective hunger to be lower when subjects knew that they were going to exercise, which may have interfered with our ability to detect true changes with exercise compared to rest. The increased consumption of fat may be important given that a reversal of the energy deficit induced by previous exercise is noted when high-fat rather than low-fat foods offered after exercise [13, 53, 54]. Finally, from our study design, it is impossible to determine whether our observed differences between running and walking are due to exercise mode or the physiological characteristics of the walkers who were on average fatter and had a lower VO_2max (i.e., were less fit) then the runners. Although the current study did not measure any long-acting adiposity hormone such as leptin or insulin, it is possible that these hormones were higher in the walkers. Emerging evidence suggests that long- and short-acting signals interact to alter hypothalamic sensitivity to satiation signals [37] which could ultimately influence eating behavior following exercise. Future studies should consider different modes of exercise along with sex and adiposity differences of the exerciser and measurement of short- and long-acting satiety signals.

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References

[1] Unites States Department of Health and Human Services, *Physical Activity Fundamental to Preventing Disease*, Unites States Department of Health and Human Services, May 2008.

[2] R. R. Pate, M. Pratt, S. N. Blair et al., "Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine," *Journal of the American Medical Association*, vol. 273, no. 5, pp. 402–407, 1995.

[3] J. M. Jakicic and A. D. Otto, "Treatment and prevention of obesity: what is the role of exercise?" *Nutrition Reviews*, vol. 64, no. 1, pp. S57–S61, 2006.

[4] U.S. Department of Health and Human Services, *Physical Activity Guidelines for Americans*, U.S. Department of Health and Human Services, Washington, DC, USA, 2008, http://www.health.gov/PAGuidelines/guidelines/default.aspx.

[5] J. M. Jakicic, K. Clark, E. Coleman et al., "Appropriate intervention strategies for weight loss and prevention of weight regain for adults," *Medicine and Science in Sports and Exercise*, vol. 33, no. 12, pp. 2145–2156, 2001.

[6] W. C. Miller, D. M. Koceja, and E. J. Hamilton, "A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention," *International Journal of Obesity*, vol. 21, no. 10, pp. 941–947, 1997.

[7] J. E. Donnelly, J. O. Hill, D. J. Jacobsen et al., "Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the midwest exercise trial," *Archives of Internal Medicine*, vol. 163, no. 11, pp. 1343–1350, 2003.

[8] T. S. Church, C. K. Martin, A. M. Thompson, C. P. Earnest, C. R. Mikus, and S. N. Blair, "Changes in weight, waist circumference and compensatory responses with different doses of exercise among sedentary, overweight postmenopausal women," *PLoS ONE*, vol. 4, no. 2, Article ID e4515, 2009.

[9] J. E. Blundell, R. J. Stubbs, D. A. Hughes, S. Whybrow, and N. A. King, "Cross talk between physical activity and appetite control: does physical activity stimulate appetite?" *Proceedings of the Nutrition Society*, vol. 62, no. 3, pp. 651–661, 2003.

[10] N. A. King, A. Tremblay, and J. E. Blundell, "Effects of exercise on appetite control: implications for energy balance," *Medicine and Science in Sports and Exercise*, vol. 29, no. 8, pp. 1076–1089, 1997.

[11] T. A. Hagobian, C. G. Sharoff, B. R. Stephens et al., "Effects of exercise on energy-regulating hormones and appetite in men and women," *American Journal of Physiology*, vol. 296, no. 2, pp. R233–R242, 2009.

[12] N. A. King, V. J. Burley, and J. E. Blundell, "Exercise-induced suppression of appetite: effects on food intake and implications for energy balance," *European Journal of Clinical Nutrition*, vol. 48, no. 10, pp. 715–724, 1994.

[13] N. A. King, L. Snell, R. D. Smith, and J. E. Blundell, "Effects of short-term exercise on appetite responses in unrestrained females," *European Journal of Clinical Nutrition*, vol. 50, no. 10, pp. 663–667, 1996.

[14] D. A. Thompson, L. A. Wolfe, and R. Eikelboom, "Acute effects of exercise intensity on appetite in young men," *Medicine and Science in Sports and Exercise*, vol. 20, no. 3, pp. 222–227, 1988.

[15] P. Imbeault, S. Saint-Pierre, N. Alméras, and A. Tremblay, "Acute effects of exercise on energy intake and feeding behaviour," *British Journal of Nutrition*, vol. 77, no. 4, pp. 511–521, 1997.

[16] S. Y. Ueda, T. Yoshikawa, Y. Katsura, T. Usui, and S. Fujimoto, "Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise," *Journal of Endocrinology*, vol. 203, no. 3, pp. 357–364, 2009.

[17] D. R. Broom, R. L. Batterham, J. A. King, and D. J. Stensel, "Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males," *American Journal of Physiology*, vol. 296, no. 1, pp. R29–R35, 2009.

[18] J. A. King, L. K. Wasse, D. R. Broom, and D. J. Stensel, "Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin," *Medicine and Science in Sports and Exercise*, vol. 42, no. 3, pp. 485–492, 2010.

[19] P. Verger, M. T. Lanteaume, J. F. Gournay, and J. Louis-Sylvestre, "Choix alimentaire spontane apres un exercise physique de natation," *Nutrition in Medicine*, vol. 28, pp. 73–77, 1992.

[20] P. Verger, M. T. Lanteaume, and J. Louis-Sylvestre, "Free food choice after acute exercise in men," *Appetite*, vol. 22, no. 2, pp. 159–164, 1994.

[21] H. R. Kissileff, F. X. Pi-Sunyer, K. Segal, S. Meltzer, and P. A. Foelsch, "Acute effects of exercise on food intake in obese and nonobese women," *American Journal of Clinical Nutrition*, vol. 52, no. 2, pp. 240–245, 1990.

[22] D. R. Broom, D. J. Stensel, N. C. Bishop, S. F. Burns, and M. Miyashita, "Exercise-induced suppression of acylated ghrelin

in humans," *Journal of Applied Physiology*, vol. 102, no. 6, pp. 2165–2171, 2007.

[23] S. F. Burns, D. R. Broom, M. Miyashita, C. Mundy, and D. J. Stensel, "A single session of treadmill running has no effect on plasma total ghrelin concentrations," *Journal of Sports Sciences*, vol. 25, no. 6, pp. 635–642, 2007.

[24] E. R. Christ, M. Zehnder, C. Boesch et al., "The effect of increased lipid intake on hormonal responses during aerobic exercise in endurance-trained men," *European Journal of Endocrinology*, vol. 154, no. 3, pp. 397–403, 2006.

[25] J. A. Cooper, A. C. Watras, C. M. Paton, F. H. Wegner, A. K. Adams, and D. A. Schoeller, "Impact of exercise and dietary fatty acid composition from a high-fat diet on markers of hunger and satiety," *Appetite*, vol. 56, no. 1, pp. 171–178, 2011.

[26] J. A. King, M. Miyashita, L. K. Wasse, and D. J. Stensel, "Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin," *Appetite*, vol. 54, no. 3, pp. 492–498, 2010.

[27] C. Martins, L. M. Morgan, S. R. Bloom, and M. D. Robertson, "Effects of exercise on gut peptides, energy intake and appetite," *Journal of Endocrinology*, vol. 193, no. 2, pp. 251–258, 2007.

[28] R. R. Russel, K. S. Willis, E. Ravussin, and E. D. Larson-Meyer, "Effect of endurance running and dietary fat on circulating ghrelin and PYY: potential role in appetite regulation," *Journal of Sports Science and Medicine*, vol. 8, no. 4, pp. 574–583, 2009.

[29] D. E. Larson-Meyer, *American College of Sports Medicine Guidelines for Exercise Testing and Prescription*, Lippincott Williams & Wilkins, Baltimore, Md, USA, 2010.

[30] H. Tanaka, K. D. Monahan, and D. R. Seals, "Age-predicted maximal heart rate revisited," *Journal of the American College of Cardiology*, vol. 37, no. 1, pp. 153–156, 2001.

[31] D. E. Larson-Meyer, K. S. Willis, L. M. Willis et al., "Effect of honey versus sucrose on appetite, appetite-regulating hormones, and postmeal thermogenesis," *Journal of the American College of Nutrition*, vol. 29, no. 5, pp. 482–493, 2010.

[32] A. Flint, A. Raben, J. E. Blundell, and A. Astrup, "Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies," *International Journal of Obesity*, vol. 24, no. 1, pp. 38–48, 2000.

[33] C. Martins, L. Morgan, and H. Truby, "A review of the effects of exercise on appetite regulation: an obesity perspective," *International Journal of Obesity*, vol. 32, no. 9, pp. 1337–1347, 2008.

[34] D. B. Dill and D. L. Costill, "Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration," *Journal of Applied Physiology*, vol. 37, no. 2, pp. 247–248, 1974.

[35] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, and K. Kangawa, "Ghrelin is a growth-hormone-releasing acylated peptide from stomach," *Nature*, vol. 402, no. 6762, pp. 656–660, 1999.

[36] D. E. Cummings, D. S. Weigle, R. Scott Frayo et al., "Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery," *The New England Journal of Medicine*, vol. 346, no. 21, pp. 1623–1630, 2002.

[37] D. E. Cummings and J. Overduin, "Gastrointestinal regulation of food intake," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 13–23, 2007.

[38] E. Ravussin, M. Tschöp, S. Morales, C. Bouchard, and M. L. Heiman, "Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 9, pp. 4547–4551, 2001.

[39] A. M. Wren, L. J. Seal, M. A. Cohen et al., "Ghrelin enhances appetite and increases food intake in humans," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 12, pp. 5992–5995, 2001.

[40] K. G. Murphy, W. S. Dhillo, and S. R. Bloom, "Gut peptides in the regulation of food intake and energy homeostasis," *Endocrine Reviews*, vol. 27, no. 7, pp. 719–727, 2006.

[41] H. Hosoda, K. Doi, N. Nagaya et al., "Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples," *Clinical Chemistry*, vol. 50, no. 6, pp. 1077–1080, 2004.

[42] K. J. Mackelvie, G. S. Meneilly, D. Elahi, A. C. K. Wong, S. I. Barr, and J. P. Chanoine, "Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 2, pp. 648–654, 2007.

[43] J. A. King, L. K. Wasse, and D. J. Stensel, "The acute effects of swimming on appetite, food intake, and plasma acylated ghrelin," *Journal of Obesity*, vol. 2011, Article ID 351628, 8 pages, 2011.

[44] E. T. Vestergaard, R. Dall, K. H. W. Lange, M. Kjaer, J. S. Christiansen, and J. O. L. Jorgensen, "The ghrelin response to exercise before and after growth hormone administration," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 1, pp. 297–303, 2007.

[45] M. Russell and M. Misra, "Influence of ghrelin and adipocytokines on bone mineral density in adolescent female athletes with amenorrhea and eumenorrheic athletes," *Medicine and Sport Science*, vol. 55, pp. 103–113, 2010.

[46] J. Erdmann, R. Tahbaz, F. Lippl, S. Wagenpfeil, and V. Schusdziarra, "Plasma ghrelin levels during exercise—effects of intensity and duration," *Regulatory Peptides*, vol. 143, no. 1–3, pp. 127–135, 2007.

[47] T. E. Adrian, G. L. Ferri, and A. J. Bacarese-Hamilton, "Human distribution and release of a putative new gut hormone, peptide YY," *Gastroenterology*, vol. 89, no. 5, pp. 1070–1077, 1985.

[48] M. R. Druce, C. J. Small, and S. R. Bloom, "Minireview: gut peptides regulating satiety," *Endocrinology*, vol. 145, no. 6, pp. 2660–2665, 2004.

[49] R. L. Batterham, M. A. Cohen, S. M. Ellis et al., "Inhibition of food intake in obese subjects by peptide YY3-36," *The New England Journal of Medicine*, vol. 349, no. 10, pp. 941–948, 2003.

[50] A. Flint, A. Raben, A. K. Ersbøll, J. J. Holst, and A. Astrup, "The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity," *International Journal of Obesity*, vol. 25, no. 6, pp. 781–792, 2001.

[51] N. M. Neary, C. J. Small, M. R. Druce et al., "Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively," *Endocrinology*, vol. 146, no. 12, pp. 5120–5127, 2005.

[52] S. Y. Ueda, T. Yoshikawa, Y. Katsura, T. Usui, H. Nakao, and S. Fujimoto, "Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males," *Journal of Endocrinology*, vol. 201, no. 1, pp. 151–159, 2009.

[53] N. A. King and J. E. Blundell, "High-fat foods overcome the energy expenditure induced by high-intensity cycling or running," *European Journal of Clinical Nutrition*, vol. 49, no. 2, pp. 114–123, 1995.

[54] A. Lluch, N. A. King, and J. E. Blundell, "Exercise in dietary restrained women: no effect on energy intake but change in hedonic ratings," *European Journal of Clinical Nutrition*, vol. 52, no. 4, pp. 300–307, 1998.

[55] M. Pomerleau, P. Imbeault, T. Parker, and E. Doucet, "Effects of exercise intensity on food intake and appetite in women," *American Journal of Clinical Nutrition*, vol. 80, no. 5, pp. 1230–1236, 2004.

[56] W. E. Reger and T. G. Allison, Eds., *Exercise, Post-Exercise Metabolic Rate and Appetite*, Human Kinetics Publishers; INC, Champaign, Ill, USA, 1984.

[57] P. Hubert, N. A. King, and J. E. Blundell, "Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity," *Appetite*, vol. 31, no. 1, pp. 9–19, 1998.

[58] R. Mattes, "Hunger ratings are not a valid proxy measure of reported food intake in humans," *Appetite*, vol. 15, no. 2, pp. 103–113, 1990.