Interindividual Responses of Appetite to Acute Exercise: A Replicated Crossover Study

FERNANDA R. GOLTZ[1,2], ALICE E. THACKRAY[1,2], JAMES A. KING[1,2], JAMES L. DORLING[1,2], GREG ATKINSON[3], and DAVID J. STENSEL[1,2]

[1]National Centre for Sport and Exercise Medicine, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UNITED KINGDOM; [2]National Institute for Health Research Leicester Biomedical Research Centre, Leicester, UNITED KINGDOM; and [3]Health and Social Care Institute, School of Health and Social Care, Teesside University, Middlesbrough, UNITED KINGDOM

ABSTRACT

GOLTZ, F. R., A. E. THACKRAY, J. A. KING, J. L. DORLING, G. ATKINSON, and D. J. STENSEL. Interindividual Responses of Appetite to Acute Exercise: A Replicated Crossover Study. *Med. Sci. Sports Exerc.*, Vol. 50, No. 4, pp. 758–768, 2018. **Purpose**: Acute exercise transiently suppresses appetite, which coincides with alterations in appetite-regulatory hormone concentrations. Individual variability in these responses is suspected, but replicated trials are needed to quantify them robustly. We examined the reproducibility of appetite and appetite-regulatory hormone responses to acute exercise and quantified the individual differences in responses. **Methods**: Fifteen healthy, recreationally active men completed two control (60-min resting) and two exercise (60-min fasted treadmill running at 70% peak oxygen uptake) conditions in randomized sequences. Perceived appetite and circulating concentrations of acylated ghrelin and total peptide YY (PYY) were measured immediately before and after the interventions. Interindividual differences were explored by correlating the two sets of response differences between exercise and control conditions. Within-participant covariate-adjusted linear mixed models were used to quantify participant–condition interactions. **Results**: Compared with control, exercise suppressed mean acylated ghrelin concentrations and appetite perceptions (all ES = 0.62–1.47, $P < 0.001$) and elevated total PYY concentrations (ES = 1.49, $P < 0.001$). For all variables, the standard deviation of the change scores was substantially greater in the exercise versus control conditions. Moderate-to-large positive correlations were observed between the two sets of control-adjusted exercise responses for all variables ($r = 0.54$–0.82, $P \leq 0.036$). After adjusting for baseline measurements, participant–condition interactions were present for all variables ($P \leq 0.053$). **Conclusions**: Our replicated crossover study allowed, for the first time, the interaction between participant and acute exercise response in appetite parameters to be quantified. Even after adjustment for individual baseline measurements, participants demonstrated individual differences in perceived appetite and hormone responses to acute exercise bouts beyond any random within-subject variability over time. **Key Words**: APPETITE, EXERCISE, GHRELIN, INDIVIDUAL DIFFERENCES, PEPTIDE YY

Understanding the relationship between exercise and appetite control has direct implications regarding the role of exercise in regulating energy homeostasis and

weight control (1,2). It is well documented that circulating concentrations of acylated ghrelin are suppressed, and satiety hormones, most notably peptide YY (PYY), are elevated in response to acute bouts of moderate- to high-intensity exercise (3). These hormonal fluctuations coincide with a transient reduction in appetite during and immediately after exercise without stimulating compensatory increases in appetite and *ad libitum* energy intake in the short term (4,5).

The notion of interindividual variability in response to an intervention, within the context of "personalized" or "precision" medicine, continues to attract significant scientific attention (6–8). Although the majority of researchers have focused on main effects and mean group changes, some investigators have attempted to quantify the individual variability in appetite and energy intake responses to acute (9–11) and chronic (12,13) exercise interventions. Some researchers have classified individuals as "compensators" or "noncompensators" according to the individual magnitude and direction of change in energy intake they observed after exercise (9,10). Although the important issue of interindividual variability has been considered in exercise and appetite regulation studies, recent

APPLIED SCIENCES

evidence has recognized that the methodological and statistical approaches for such investigations are challenging and often lacking in some cases (6,14,15).

One approach to quantifying "true" individual responses is via the participant–response interaction term in a statistical model, which requires replicated intervention and comparator arms with sufficient washout (16,17). Previous researchers have reported intraclass coefficients to support claims that pre-to-post changes in *ad libitum* energy intake in response to acute exercise are not consistent within an individual over time (11,18). Interindividual variability in appetite and appetite-regulatory hormone responses to repeated acute exercise exposures are suspected; however, no published studies have confirmed this notion using robust designs (the replicated crossover) and appropriate statistical models.

Therefore, the aims of the present study were to examine the reproducibility of appetite, acylated ghrelin, and total PYY responses to acute exercise bouts and to quantify the magnitude of individual differences in responses using a replicated crossover design. Recent insights have provided a framework for the accurate statistical analyses to quantify true interindividual variability in exercise responses using the standard deviation (SD) of the change scores and participant–response interaction (6,14–17). Using these approaches, it was hypothesized that exercise-induced changes in subjective and hormonal appetite parameters would be reproducible on repeated occasions, and true interindividual variability in appetite responses to acute exercise bouts would be observed in healthy, recreationally active men.

METHODS

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki (2013), and all procedures were approved by the local ethics advisory committee. All participants provided written informed consent before taking part in any aspect of the study.

Participants

Fifteen healthy, recreationally active men (mean [SD]: age, 23 [3] yr; body mass, 81.9 [11.4] kg; body mass index, 24.8 [3.0] $kg \cdot m^{-2}$; waist circumference, 84.3 [6.9] cm; body fat percentage, 13.1 [5.9]%; peak oxygen uptake [$\dot{V}O_2$], 54.9 [6.5] $mL \cdot kg^{-1} \cdot min^{-1}$) participated in the study. The participants' body mass was stable; ≤3 kg (≤3.7%) change in the previous 3 months. Participants were nonsmokers, had no history of cardiovascular or metabolic disease, and were not dieting or taking any medications.

Preliminary Measurements

Before the main experimental conditions, participants attended the laboratory for a preliminary visit to complete screening questionnaires and to undergo familiarization, anthropometric measurements, and exercise testing. Specifically, participants completed questionnaires assessing health status, food preferences, habitual physical activity (International Physical Activity Questionnaire) (19), and psychological eating tendencies (Three-Factor Eating Questionnaire) (20). Height and body mass were quantified using an electronic measuring station (Seca, Hamburg, Germany). Waist circumference was measured at the narrowest point of the torso between the lower rib margin and the iliac crest. The sum of seven skinfolds was used to estimate body density (21) and body fat percentage (22).

After familiarization with walking and running on the treadmill (Excite Med; Technogym, Cesena, Italy), participants completed two preliminary exercise tests. The first test involved a 16-min submaximal incremental treadmill protocol divided into 4 × 4-min stages to determine the relationship between treadmill speed and oxygen consumption. The initial running speed was set between 8 and 12 $km \cdot h^{-1}$ depending on each participant's fitness level, and the treadmill speed was increased by 1 to 1.5 $km \cdot h^{-1}$ at the start of each subsequent stage. Heart rate was monitored continuously using short-range telemetry (Polar A3; Polar, Kempele, Finland), and ratings of perceived exertion (RPE) (23) were assessed at the end of each stage. Expired air samples were collected into Douglas bags in the final minute of each 4-min stage. Oxygen consumption and carbon dioxide production were determined using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1400; Servomex, East Sussex, UK), and the volume of expired air was quantified using a dry gas meter (Harvard Apparatus, Kent, UK).

After a 20-min standardized rest period, peak $\dot{V}O_2$ was measured using an incremental uphill treadmill protocol at a constant speed until the participants reached volitional fatigue. The initial incline of the treadmill was set at 3.5% which was increased by 2.5% every 3 min (24). Peak $\dot{V}O_2$ was determined from an expired air sample collected in the final minute when participants indicated that they could only continue for an additional 1 min. Heart rate and RPE were monitored throughout the tests as described previously. Data from the 16-min submaximal incremental and peak $\dot{V}O_2$ tests were used to determine the running speed required to elicit 70% of peak $\dot{V}O_2$ during the experimental exercise conditions.

Experimental Design

In a replicated, crossover experimental design, participants were randomized to different sequences of four experimental conditions: two control and two exercise (17). Each condition was separated by an interval of at least 5 d. Participants completed a weighed food record in the 24 h preceding the first experimental condition and were instructed to replicate this feeding pattern before each subsequent condition. Participants refrained from alcohol, caffeine, and strenuous physical activity during the same period. A standardized meal was consumed in the evening before the experimental conditions consisting of a pepperoni pizza (4891 kJ, 48% carbohydrate, 18% protein, 34% fat). Participants were instructed to consume the meal between 19:00 and 20:00, after which they consumed no food or drink except plain water until arriving at the laboratory the next morning.

Main Trials

Participants arrived at the laboratory at 08:00 having fasted overnight for a minimum of 12 h. A cannula (Venflon; Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein for venous blood sampling, and participants rested for 1 h (~08:00–09:00) to acclimatize to the study environment (25). During both exercise conditions, participants then completed 60 min of fasted treadmill running at a speed predicted to elicit 70% of peak $\dot{V}O_2$. One minute expired air samples were collected and analyzed every 15 min, and the treadmill speed was adjusted if necessary during both exercise conditions to ensure the target exercise intensity was achieved. Heart rate was monitored continuously, and RPE was determined after each expired air sample was collected. The exercise energy expenditure and substrate utilization were subsequently estimated using the equations of Frayn (26). Identical procedures were completed during both control conditions except participants rested within the laboratory for the equivalent duration.

Appetite Perceptions

Ratings of perceived appetite (hunger, satisfaction, fullness, and prospective food consumption [PFC]) were assessed immediately before (0 h) and after (1 h) the exercise and control interventions using 100 mm visual analogue scales (27). The scales were anchored by a descriptor at each end defining the extremes of the appetite perception being measured.

Blood Sampling and Biochemical Analysis

Blood samples were collected in the semisupine position immediately before (0 h) and after (1 h) the exercise and control interventions for the assessment of plasma acylated ghrelin and total PYY concentrations. Plasma acylated ghrelin concentrations were quantified from venous blood samples collected into prechilled 4.9 mL EDTA monovettes (Sarstedt, Leicester, UK). These monovettes contained *p*-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease and were centrifuged at 2383*g* for 10 min at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was aliquoted into a storage tube, and 100 μL of 1 M hydrochloric acid was added per milliliter of plasma. Samples were recentrifuged at 2383*g* for 5 min at 4°C before being transferred into Eppendorf tubes and stored at −80°C for later analysis. Venous blood samples for plasma total PYY were collected into prechilled 4.9 mL EDTA monovettes (Sarstedt, Leicester, UK) and centrifuged at 2383*g* for 10 min at 4°C before storage at −80°C. Measurements of hemoglobin and hematocrit were determined in duplicate at 0 and 1 h in all conditions to calculate the acute change in plasma volume (28).

Commercially available enzyme immunoassays were used to determine the plasma concentrations of acylated ghrelin (SPI Bio, Montigny-le-Bretonneux, France) and total PYY (Millipore, Watford, UK). All samples were analyzed in duplicate. To eliminate interassay variation, samples for each participant were analyzed in the same run. The within-batch coefficients of variation for acylated ghrelin and total PYY concentrations were 4.1% and 3.6%, respectively.

Statistical Analyses

Data were analyzed using the IBM SPSS Statistics software for Windows version 23.0 (IBM Corporation, New York, NY) and the PROC MIXED procedure in *SAS OnDemand for Academics* (https://www.sas.com/en_us/software/on-demand-for-academics.html). The presence of interindividual differences in acylated ghrelin, total PYY, and perceived appetite responses to acute exercise bouts were examined according to three recently reported analytical approaches (6,16,17):

(i) Pearson's correlation coefficients were quantified between the exercise and control pre-to-post (0–1 h) change scores for each appetite parameter on the two occasions (17). The first exercise bout in any participant's sequence was paired to the first control bout in the same individual's sequence. Differences between these trials were correlated with the second exercise-control condition differences in the participant's trial sequence. Thresholds of 0.1, 0.3, and 0.5 were used to define small, moderate, and large correlation coefficients, respectively (29).

(ii) The difference in SDs of the pre-to-post changes between the exercise and control conditions was calculated to represent the true individual response SD using the following equation:

$$SD_R = \sqrt{SD_E^2 - SD_C^2}$$

where SD_R is the SD of the true individual response to the exercise conditions and SD_E and SD_C are the SDs of the pre-to-post change scores for the exercise and control conditions, respectively (6,15). This estimation of the true SD for individual differences in response should be considered a "naive estimation" because important aspects of the experimental design, for example, period effects, are not included. Therefore, a modeling approach to this estimation was also adopted (see iii below).

(iii) A within-participant linear mixed model was formulated to quantify any participant–condition interaction for each appetite parameter. Condition and period (sequence) were initially modeled as fixed effects. Senn et al. raised the question of whether the participant and participant–condition interaction terms should be modeled as fixed or random effects (16). Differences between these modeling approaches may exist depending on the distribution of the participant factor and the magnitude of the treatment (exercise effect). Our sample was, in clinical trial terms, relatively small, and we expected the general effects of exercise to be substantial. Therefore, we modeled our data with participant and participant–condition terms as both fixed and random effects and compared these results as a sensitivity analysis. When the participant–condition interaction

APPLIED SCIENCES

was considered as a random effect, we used the SAS code supplied by Senn et al. with a modification designed to derive the true individual response variance (also estimated by approach ii) (16). This modification involved the adding of a covariate "dummy" variable we called "XVARE" (see Document, Supplemental Digital Content 1, SAS code, http://links.lww.com/MSS/B114).

It is also relevant to explore the extent to which an individual's response depends on their status at baseline (6). Therefore, baseline status of the dependent variable was added to the various linear mixed models as a covariate. The mean differences between conditions were also quantified with this same statistical model.

We found that correction of appetite hormone concentrations for acute changes in plasma volume had a negligible influence on our findings. Therefore, the unadjusted plasma concentrations are displayed for simplicity. Absolute standardized effect sizes (ES) were calculated, with a standardized ES of 0.2 denoting the minimum important mean difference for all outcomes, 0.5 denoting moderate, and 0.8 denoting large (29). To calculate the minimal clinically important difference (MCID) for individual responses, the threshold of 0.2 for interpreting standardized mean changes (29) was halved, that is, 0.1, and multiplied by the baseline between-subject SD (6,15). Pearson correlation coefficients were quantified between the pooled mean pre-to-post change in appetite-regulatory hormone concentrations and the pooled mean pre-to-post change in appetite perceptions across the four conditions.

Data are described as mean (SD). Mean differences and correlation coefficients are presented along with respective 95% confidence intervals (95% CIs). P values are expressed in exact terms apart for very low values, which are expressed as $P < 0.001$, and statistical significance was accepted as $P < 0.05$.

RESULTS

Treadmill Exercise Responses

Treadmill exercise responses are displayed in Table 1. No statistically significant or practically important differences were observed in any of the treadmill exercise responses between the two exercise sessions ($P \geq 0.13$).

Acylated Ghrelin

A moderate positive correlation of 0.57 (95% CI, 0.08–0.84; $P = 0.025$) was observed between the two sets of control-adjusted exercise responses for acylated ghrelin (Fig. 1A). The within-trial SD for acylated ghrelin was substantially greater for the exercise than control conditions (Table 2). Baseline-adjusted linear mixed models for acylated ghrelin concentrations revealed a significant main effect of condition ($P < 0.001$) and a significant participant–condition interaction ($P < 0.001$). The mean acylated ghrelin concentration was 51 pg·mL^{-1} lower (95% CI, −59 to −43 pg·mL^{-1}; ES = 0.62) in the exercise versus control conditions. The magnitude of change in individual replicated mean responses after exercise for acylated ghrelin ranged from −141 to −9 pg·mL^{-1}, with 100% ($n = 15$) of participants demonstrating a suppression beyond the MCID (±8.20 pg·mL^{-1}) (Fig. 1B).

Total PYY

A small positive correlation of 0.27 (95% CI, −0.28 to 0.69; $P = 0.339$) was observed between the two sets of control-adjusted exercise responses for total PYY (Fig. 2A). Based on the recommendations of Hopkins et al., an outlier was identified who exhibited a PYY response greater than 3.5 residual SDs from the mean predicted value (30). After removal of the outlier, the correlation for total PYY increased to 0.71 and became significant (95% CI, 0.31–0.90; $P = 0.003$) (Fig. 2B). The within-trial SD for total PYY was substantially greater for the exercise than control conditions (Table 2). Baseline-adjusted linear mixed models for total PYY concentrations revealed a significant main effect of condition ($P < 0.001$) and a significant participant–condition interaction ($P = 0.012$). The mean total PYY concentration was 56 pg·mL^{-1} higher (95% CI, 44–68 pg·mL^{-1}; ES = 1.49) in the exercise versus control conditions. The magnitude of change in individual replicated mean responses after exercise for total PYY ranged from 3 to 112 pg·mL^{-1}, with 93% ($n = 14$) of participants demonstrating an increase beyond the MCID (±3.75 pg·mL^{-1}) (Fig. 2C).

Appetite Ratings

Moderate-to-large positive correlations were observed between the two sets of control-adjusted exercise responses for hunger ($r = 0.82$; 95% CI, 0.53–0.94; $P < 0.001$), satisfaction ($r = 0.74$; 95% CI, 0.37–0.91; $P = 0.002$), fullness ($r = 0.55$; 95% CI, 0.05–0.83; $P = 0.035$), and PFC ($r = 0.54$; 95% CI, 0.04–0.82; $P = 0.036$) (Fig. 3). The within-trial SD was substantially greater for the exercise than control conditions for hunger, satisfaction, fullness, and PFC (Table 2).

TABLE 1. The various responses during the treadmill exercise for the 2 exercise conditions.

Variable	Exercise Condition 1	Exercise Condition 2	95% CI*	ES
Oxygen uptake (mL·kg^{-1}·min^{-1})	38.9 (5.1)	38.5 (4.9)	−4.2 to 3.3	0.09
% peak oxygen uptake	71 (3)	70 (3)	−2 to 0.3	0.31
Heart rate (bpm)	176 (10)	176 (13)	−5 to 4	0.04
Rating of perceived exertion	15 (2)	15 (2)	−1 to 0.2	0.13
Respiratory exchange ratio	0.91 (0.03)	0.92 (0.04)	−0.01 to 0.02	0.21
Fat oxidation (g)	29 (12)	26 (14)	−7 to 2	0.22
Carbohydrate oxidation (g)	159 (29)	164 (36)	−6 to 15	0.13
Net energy expenditure (kJ)	3473 (551)	3433 (532)	−104 to 23	0.08

Values are mean (SD). *95% confidence interval for the mean absolute difference between exercise conditions. ES indicates standardized (to between-subjects SD) effect size.
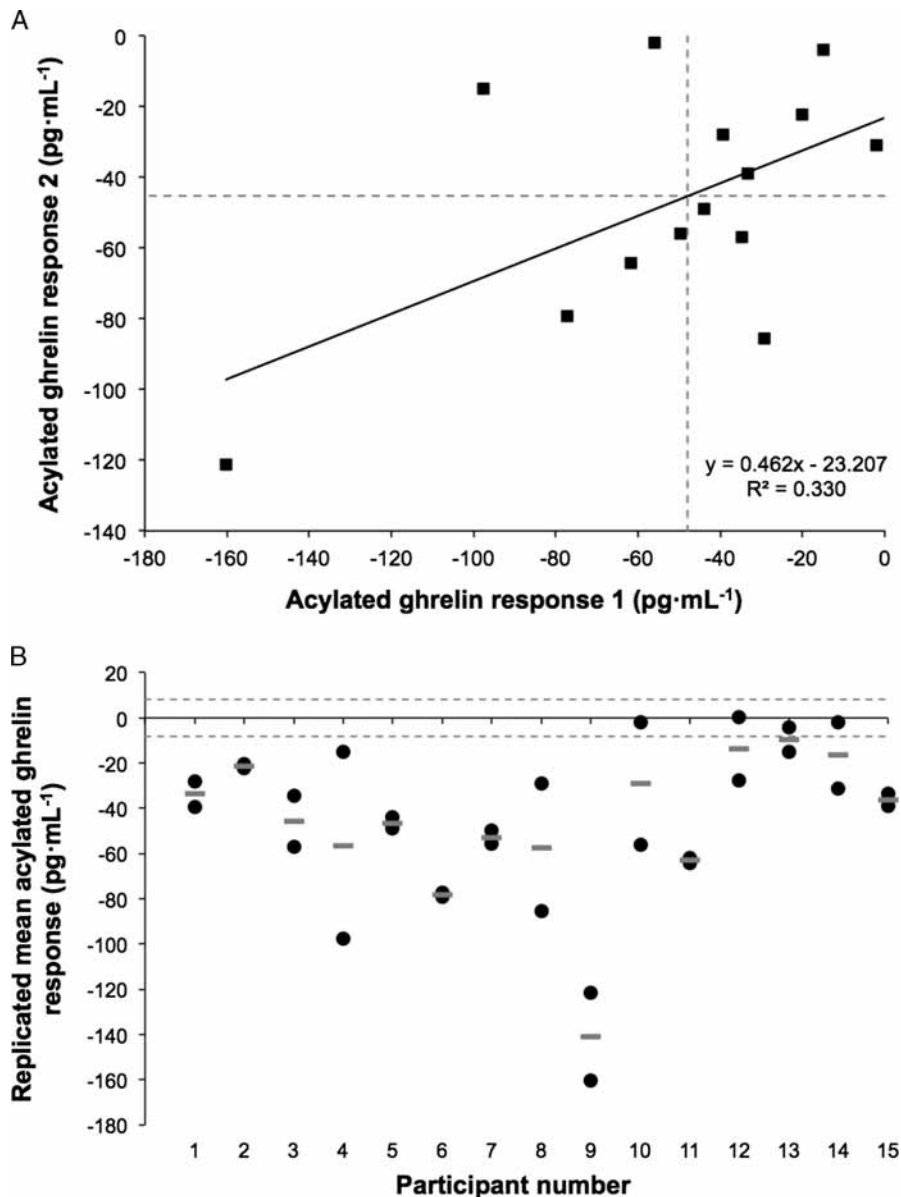
APPLIED SCIENCES

FIGURE 1—A, Relationship between exercise and control pre-to-post (0–1 h) change scores on the two occasions for acylated ghrelin. "Response 1" corresponds to the first pair of conditions (exercise 1 minus control 1) and "response 2" to the second pair of conditions (exercise 2 minus control 2). Dashed lines represent the mean responses. B, Individual changes in acylated ghrelin between the exercise and control conditions (exercise minus control). Black circles (•) indicate pre-to-post change scores for "response 1" and "response 2" for each participant. Gray lines (—) represent each participant's replicated mean response. Dashed lines indicate the standardized minimal clinically important difference calculated as 0.1 multiplied by the baseline between-subject SD (6).

Baseline-adjusted linear mixed models for all ratings of perceived appetite revealed a main effect of condition ($P < 0.001$) and participant–condition interactions ($P \leq 0.053$). The main effect of condition identified suppressed appetite in the exercise compared with control conditions. The mean ratings of hunger and PFC were 26 mm (95% CI, -29 to -22 mm; ES = 1.47) and 19 mm (95% CI, -25 to -13 mm; ES = 1.05) lower in the exercise versus control conditions, respectively. The mean ratings of satisfaction and fullness were 15 mm (95% CI, 11–20 mm; ES = 0.95) and 14 mm (95% CI, 8–21 mm; ES = 0.88) higher in the exercise versus control conditions, respectively. The magnitude of change in individual replicated

mean responses after exercise ranged from -65 to 10 mm for hunger, -13 to 72 mm for satisfaction, -23 to 89 mm for fullness, and -96 to 7 mm for PFC. Ninety-three percent ($n = 14$) of participants demonstrated a response beyond the MCID for hunger (± 1.76 mm; 13% above, 80% below) and satisfaction (± 1.62 mm; 60% above, 33% below), 87% ($n = 13$) for fullness (± 1.64 mm; 53% above, 33% below), and 100% ($n = 15$) for PFC (± 1.82 mm; 33% above, 67% below) (Fig. 4).

A sensitivity analysis with the participant factor entered into the statistical model as a random, rather than a fixed, effect also resulted in participant–condition interactions for all appetite parameters (Table 2; $P = 0.013$–0.077).

TABLE 2. Unadjusted mean and SDs of the pre-to-post change scores for the exercise and control conditions and the true individual differences SD.

Variable	Exercise Change Mean (SD)	Control Change Mean (SD)	Estimate 1 Individual Differences SD	Estimate 2 Individual Differences SD (SE)	P
Acylated ghrelin (pg·mL^{-1})	−41.9 (33.1)	4.8 (13.0)	30.4	30.9 (19.7)	0.014
Total PYY (pg·mL^{-1})	40.7 (35.5)	−10.7 (23.1)	27.0	25.7 (19.3)	0.077
Hunger (mm)	−13.6 (26.8)	10.5 (7.5)	25.7	24.5 (15.5)	0.013
Satisfaction (mm)	6.5 (25.1)	−7.7 (8.9)	23.5	23.2 (14.8)	0.015
Fullness (mm)	3.6 (34.8)	−8.3 (9.8)	33.4	31.6 (20.1)	0.013
Prospective food consumption (mm)	−9.9 (27.7)	7.7 (9.6)	26.0	23.7 (15.5)	0.019

Estimate 1: Individual differences SD estimated using $SD_R = \sqrt{SD_E^2 - SD_C^2}$ where SD_R is the SD of the true individual response, and SD_E and SD_C are the SDs of the pre-to-post change scores for the exercise and control conditions, respectively (6,15).
Estimate 2: Individual differences SD estimated using a random effects statistical model based on Senn et al. (16). The SD was derived from the SAS model participant–condition interaction term (as a random effect). The P value shown is also for this interaction term.
SE, standard error; PYY, peptide YY.

Correlations

A large positive correlation was observed between the pre-to-post change in acylated ghrelin and the change in both hunger ($r = 0.72$; 95% CI, 0.33–0.90; $P = 0.002$) and PFC ($r = 0.63$; 95% CI, 0.17–0.86; $P = 0.011$). There were no significant correlations between the pre-to-post change in PYY and appetite perceptions ($P \geq 0.129$) (see Table, Supplemental Digital Content 2, Pearson's correlation coefficients between the pooled mean pre-to-post change in appetite-regulatory hormone concentrations and the pooled mean pre-to-post change in appetite perceptions across the 4 conditions, http://links.lww.com/MSS/B115).

DISCUSSION

The primary finding from our replicated crossover trial of appetite responses to exercise was that true interindividual variability exists in the appetite, acylated ghrelin, and total PYY responses to acute exercise bouts beyond any measurement error and random within-subject variability over time. A further finding was the moderate-to-large positive correlations observed between the exercise and control pre-to-post change scores on two occasions, indicating good reproducibility for exercise-induced changes in appetite parameters.

Our study supports previous literature by confirming the appetite-suppressing effect of acute exercise (3,5). In this regard, the grand mean changes at the sample level indicated a suppression of acylated ghrelin and perceived appetite and an increase in total PYY after the exercise session. The correlation coefficients quantified between the exercise and control pre-to-post change scores on the two pairs of conditions were positive, significant, and moderate-to-large for perceived appetite and acylated ghrelin. Although the correlation for total PYY was small and nonsignificant, closer examination of the change scores revealed that one participant presented two very opposite responses to exercise. Specifically, the change score between the first pair of trials indicated a suppression in total PYY (−34 pg·mL^{-1}), and the second pair of trials showed a very strong increase in total PYY (146 pg·mL^{-1}) (Figs. 2A and C). The reason for this disparity is unclear, and removal of this apparent outlier resulted in a larger correlation of similar magnitude to the other appetite-related outcomes measured in our study. Overall, responses to exercise were similar on repeated occasions, providing evidence to support the reproducibility of changes in appetite parameters after acute exercise.

Although no previous researchers have quantified the reproducibility of perceived appetite or appetite-regulatory hormone responses to acute exercise, the reproducibility of postexercise energy intake has received more attention (11,18,31). Specifically, Laan et al. (31) reported good reproducibility for ad libitum energy intake after duplicate aerobic exercise, resistance exercise, and resting control conditions in young, active adults (31). However, the difference in ad libitum energy intake between the exercise and control conditions was not calculated in the study by Laan et al. (31). Therefore, it can be said that within-subject variations were not taken into account, and the possibility of the observed responses to exercise being exclusively due to measurement errors and random variability cannot be excluded (6,15). Although energy intake seems reproducible when considering repeated resting and exercise conditions in isolation (11,31), the reproducibility of the difference in ad libitum energy intake between exercise and control interventions appears low when assessed with the use of intraclass coefficients (11,18).

Alongside the good reproducibility of appetite responses to acute exercise, our data show that individuals differ in the general magnitude of this response (the mean of the replicated trials, Figs. 1B, 2C, and 4). A statistically significant participant–condition interaction was observed for all appetite parameters, even after adjusting for baseline values. Although previous studies have reported individual variability in perceived appetite and energy intake responses to acute exercise in healthy (9) and overweight and obese women (10), this variability was estimated using a single pair of trials, that is, one control and one exercise condition. Repeated administrations of treatment in a crossover fashion with a comparator arm (control condition) are required to assess individual variability in response to short-term or acute interventions from the participant–condition interaction term (15). We are not aware of previous studies assessing individual variability in appetite and appetite-regulatory hormone responses to acute exercise using a replicated crossover design and the statistical methods used in the present study.
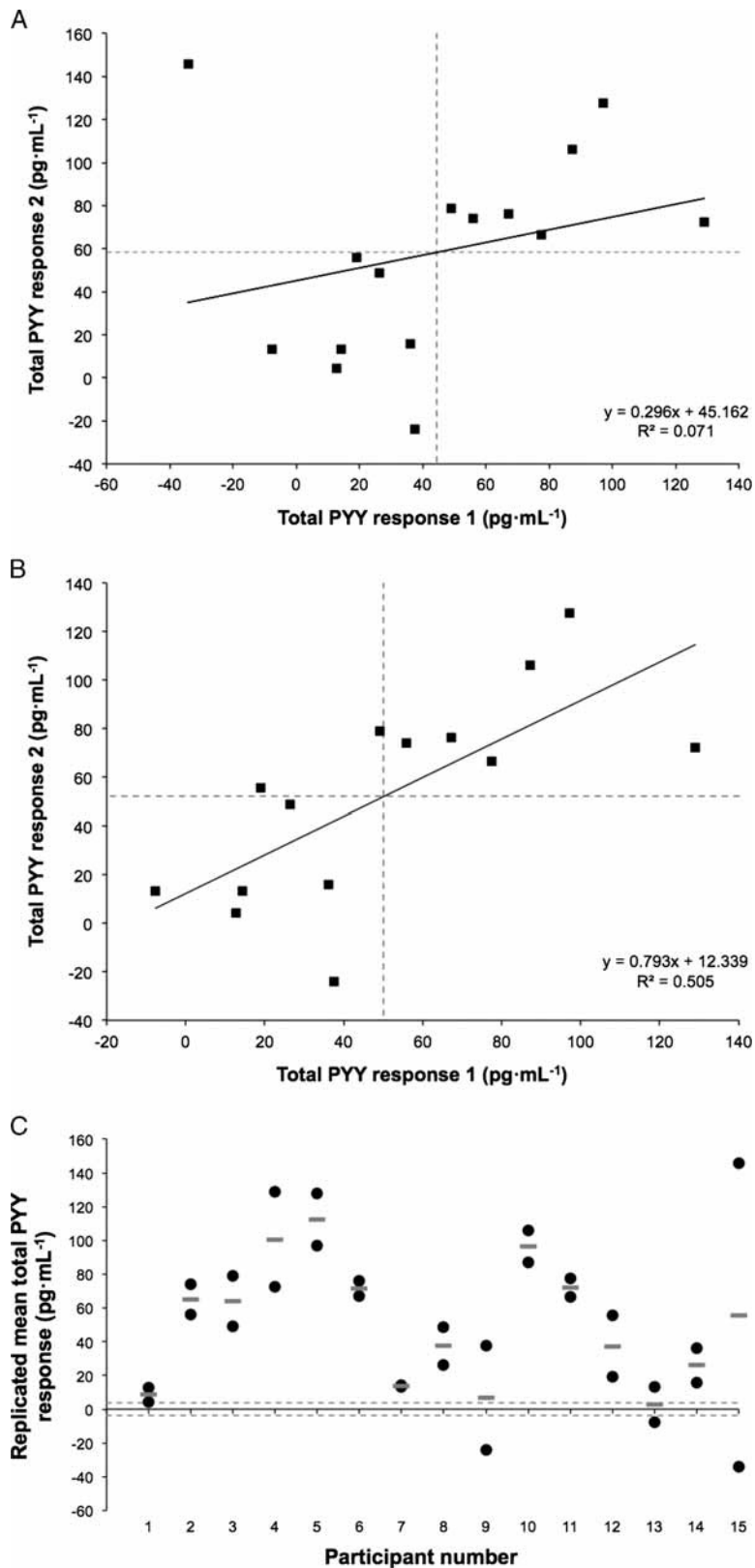
APPLIED SCIENCES

FIGURE 2—Relationship between exercise and control pre-to-post (0–1 h) change scores on the two occasions for total PYY before (A) and after (B) the removal of a substantial outlier. "Response 1" corresponds to the first pair of conditions (exercise 1 minus control 1) and "response 2" to the second pair of conditions (exercise 2 minus control 2). Dashed lines represent the mean responses. C, Individual changes in total PYY between the exercise and control conditions (exercise minus control). Black circles (●) indicate pre-to-post change scores for "response 1" and "response 2" for each participant. Gray lines (−) represent each participant's replicated mean response. Dashed lines indicate the standardized minimal clinically important difference calculated as 0.1 multiplied by the baseline between-subject SD (6).
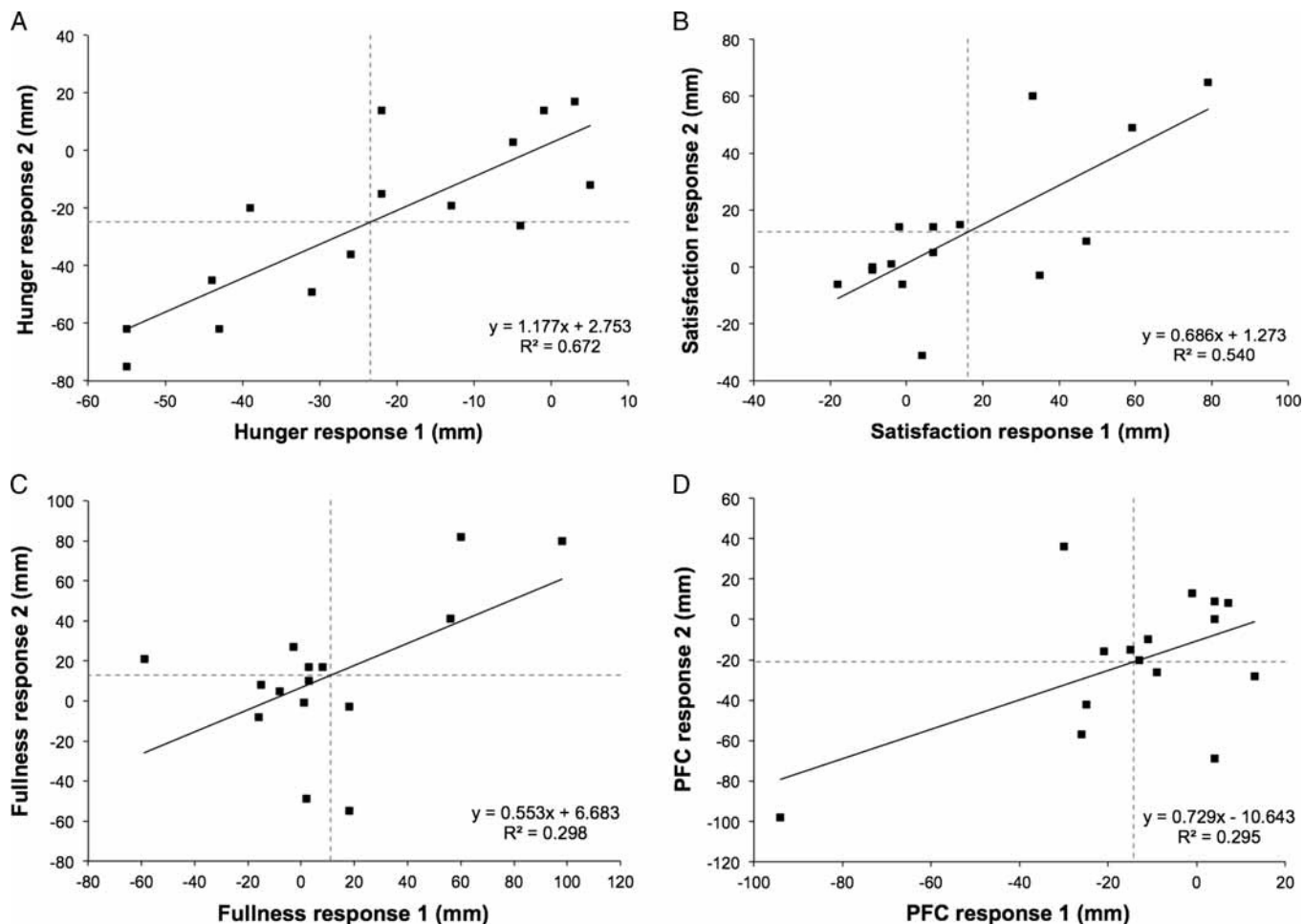
APPLIED SCIENCES

FIGURE 3—Relationship between exercise and control pre-to-post (0–1 h) change scores on the two occasions for (A) hunger, (B) satisfaction, (C) fullness, and (D) prospective food consumption (PFC). "Response 1" corresponds to the first pair of conditions (exercise 1 minus control 1) and "response 2" to the second pair of conditions (exercise 2 minus control 2). Dashed lines represent the mean responses.

The SD of the change scores is a good indication of individual variability in the responses to an intervention. If the SD of the change scores does not differ substantially between control and intervention conditions, the change originated by the intervention could be explained by random within-subject variation and measurement error (6,15). The true individual response SD (using both estimates 1 and 2) was relatively large compared with the mean response for all appetite-related variables measured in this study (Table 2). For example, although the mean unadjusted exercise response (versus control change) for acylated ghrelin was approximately 47 $pg \cdot mL^{-1}$, the true individual response SD was approximately ± 30 $pg \cdot mL^{-1}$ (Table 2). This SD indicates the presence of substantial true interindividual differences in the acylated ghrelin response to exercise; this interpretation also applies to the other appetite parameters we assessed.

Furthermore, we also highlight that most participants showed appetite responses that exceeded the MCID we selected. Therefore, very few participants were identified as "nonresponders," but some were "very large responders," whereas others were "small responders" according to the

magnitude of change in acylated ghrelin, total PYY, and appetite perceptions after single bouts of exercise (Figs. 1B, 2C, and 4). Specifically, all participants demonstrated replicated mean responses beyond the MCID for circulating acylated ghrelin indicating an exercise-induced suppression of this hormone, and 93% of participants experienced an increase in circulating total PYY beyond the MCID. The direction of the replicated mean responses was more variable for the perceived appetite ratings. Of the participants that demonstrated replicated mean responses beyond the MCID, 53% to 80% of participants reported suppressed appetite after exercise (ie, lower hunger and PFC, higher satisfaction and fullness), whereas 13% to 33% of participants reported higher perceived appetite after exercise (ie, higher hunger and PFC, lower satisfaction and fullness).

Although some studies report concomitant changes in appetite-regulatory hormones and appetite perceptions in response to acute exercise at the group level (32,33), exercise-induced changes in these parameters do not always occur simultaneously (34–36). The present study extends these findings by demonstrating that most participants exhibited corresponding exercise-induced changes in acylated
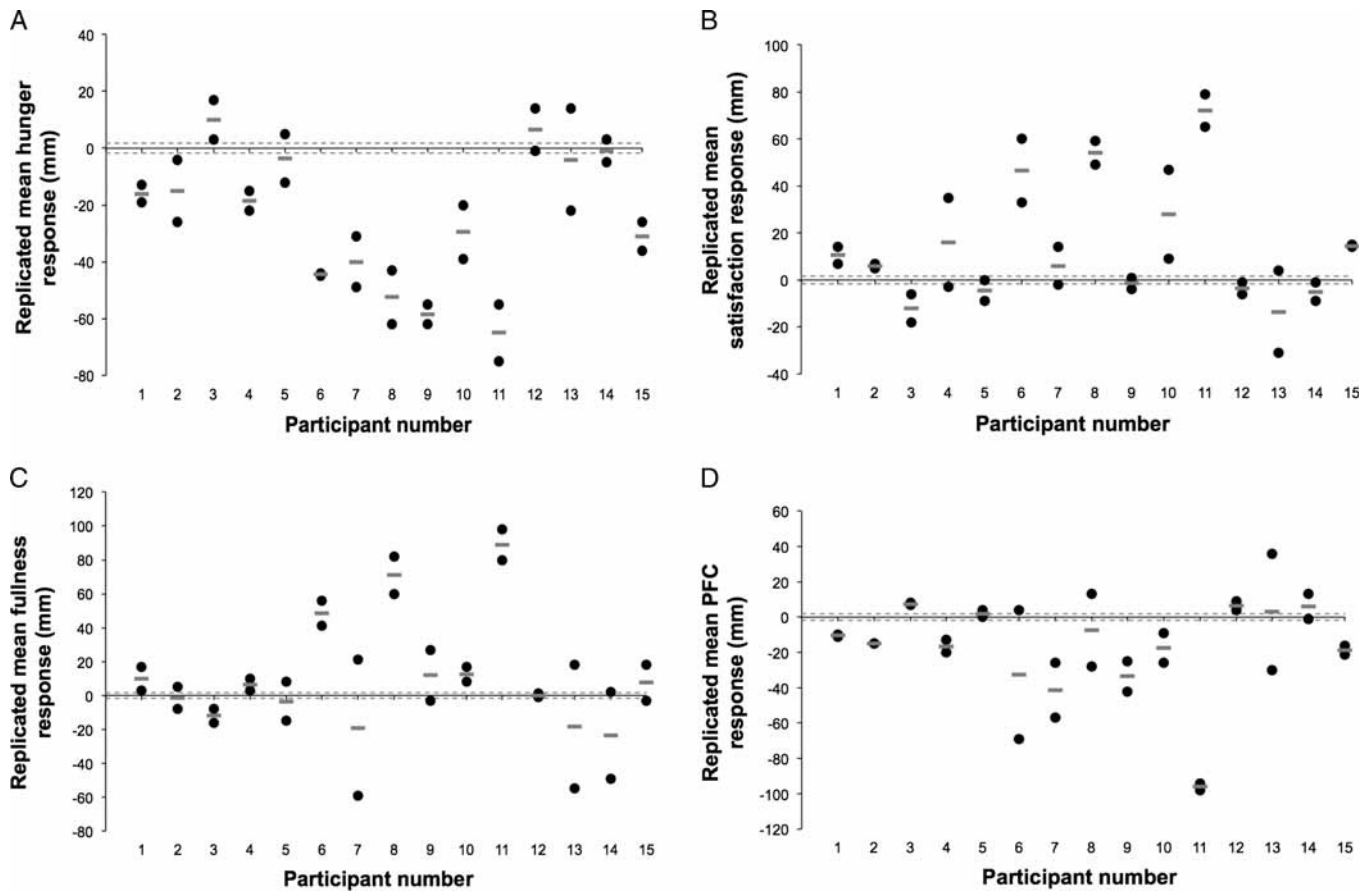
APPLIED SCIENCES

FIGURE 4—Individual changes in each perceived appetite rating between the exercise and control conditions (exercise minus control): (A) hunger, (B) satisfaction, (C) fullness, and (D) prospective food consumption (PFC). Black circles (•) indicate pre-to-post change scores for "response 1" and "response 2" for each participant. Gray lines (–) represent each participant's replicated mean response. Dashed lines indicate the standardized minimal clinically important difference calculated as 0.1 multiplied by the baseline between-subject SD (6).

ghrelin, total PYY, and appetite perceptions and is further supported by the meaningful positive relationships observed between the pre-to-post change in acylated ghrelin and the change in hunger and PFC. However, some participants demonstrated divergent subjective and hormonal appetite responses to exercise. It is well established that appetite regulation is a complex process involving the interaction of many physiological and psychological factors (1). Therefore, perceived appetite in some participants could have been more strongly affected by other variables not assessed in the present study. In this regard, several other anorexigenic gut peptides are involved in the acute regulation of appetite including cholecystokinin, oxyntomodulin, pancreatic polypeptide, and glucagon-like peptide-1. Indeed, the absence of significant correlations between the pre-to-post change in total PYY and appetite perceptions may reflect the notion that PYY acts synergistically with these other satiety signals to suppress appetite. Furthermore, appetite control is influenced by a variety of nonhomeostatic factors such as neuronal responses, hedonic processes, and cognitive/behavioral cues (37). Future studies should consider the aforementioned appetite parameters to provide a more holistic scientific understanding of the variability in appetite responses after acute exercise.

A potential source of variability in this study concerns the measurement of acylated ghrelin and total PYY concentrations from venous blood samples collected from an antecubital vein. Recent studies suggest that compared to arterialized blood, venous blood provides lower concentrations of glucagon-like peptide-1 (38) as well as lower glucose concentrations and higher insulin sensitivity (39). Although limited evidence in patient populations suggests that fasting ghrelin concentrations are comparable between venous and arterialized blood (40,41), direct comparisons of acylated ghrelin and total PYY between arterialized and venous blood after exercise has not been investigated. Nevertheless, the findings of the present study are relevant to the wider exercise and appetite regulation literature where blood sampling from an antecubital vein is commonplace for quantifying appetite-regulatory hormone concentrations.

The strengths of our study include the replicated cross-over design and the use of recently published robust statistical analyses for individual variability quantification. Moreover, the detailed standardization protocol followed by all participants during the 24 h preceding each laboratory visit and the precise replication of the exercise sessions add credibility to our results. However, it should be highlighted that

our results cannot be generalized to other populations such as female subjects, overweight or obese, and older individuals who may present different results (42,43). It is also possible that different exercise modes, intensities, or session durations would elicit different responses (5,34,44). Therefore, further research is needed to assess the reproducibility and individual variability of exercise-induced changes in appetite-regulatory hormones and appetite perceptions in other populations and with different exercise protocols. The publication of more studies investigating individual variability in appetite responses to exercise may stimulate the development of more efficient weight management strategies by determining whether an exercise intervention is likely to be beneficial, ineffective, or detrimental for different individuals. This information would help to identify individuals who may achieve more favorable appetite responses through alternative exercise and/or nutritional interventions, but further work is required to examine this chronically.

In conclusion, healthy, young men exhibited reproducible appetite responses to acute exercise, and true individual variability exists in acylated ghrelin, total PYY, and perceived appetite responses over and above any random within-subject variability and measurement error. Individual variability in appetite responses to acute exercise needs to be considered when interpreting study results so that misleading conclusions can be avoided.

REFERENCES

1. Stensel D. Exercise, appetite and appetite-regulating hormones: implications for food intake and weight control. *Ann Nutr Metab*. 2010;57(2 Suppl):36–42.
2. Beaulieu K, Hopkins M, Blundell J, Finlayson G. Does habitual physical activity increase the sensitivity of the appetite control system? A systematic review. *Sports Med*. 2016;46(12):1897–919.
3. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Med*. 2014;44(3):387–403.
4. Schubert MM, Desbrow B, Sabapathy S, Leveritt M. Acute exercise and subsequent energy intake. A meta-analysis. *Appetite*. 2013;63:92–104.
5. Deighton K, Stensel DJ. Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? *Proc Nutr Soc*. 2014;73(2):352–8.
6. Atkinson G, Batterham AM. True and false interindividual differences in the physiological response to an intervention. *Exp Physiol*. 2015;100(6):577–88.
7. Betts JA, Gonzalez JT. Personalised nutrition: what makes you so special? *Nutr Bull*. 2016;41(4):353–9.
8. King JA, Deighton K, Broom DR, et al. Individual variation in hunger, energy intake, and ghrelin responses to acute exercise. *Med Sci Sports Exerc*. 2017;49(6):1219–28.
9. Finlayson G, Bryant E, Blundell JE, King NA. Acute compensatory eating following exercise is associated with implicit hedonic wanting for food. *Physiol Behav*. 2009;97(1):62–7.
10. Hopkins M, Blundell JE, King NA. Individual variability in compensatory eating following acute exercise in overweight and obese women. *Br J Sports Med*. 2014;48(20):1472–6.
11. Unick JL, O'Leary KC, Dorfman L, Thomas JG, Strohacker K, Wing RR. Consistency in compensatory eating responses following acute exercise in inactive, overweight and obese women. *Br J Nutr*. 2015;113(7):1170–7.
12. Barwell ND, Malkova D, Leggate M, Gill JM. Individual responsiveness to exercise-induced fat loss is associated with change in resting substrate utilization. *Metabolism*. 2009;58(9):1320–8.
13. King NA, Caudwell PP, Hopkins M, Stubbs JR, Naslund E, Blundell JE. Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *Am J Clin Nutr*. 2009;90(4):921–7.
14. Hecksteden A, Kraushaar J, Scharhag-Rosenberger F, Theisen D, Senn S, Meyer T. Individual response to exercise training—a statistical perspective. *J Appl Physiol (1985)*. 2015;118(12):1450–9.
15. Hopkins WG. Individual responses made easy. *J Appl Physiol (1985)*. 2015;118(12):1444–6.
16. Senn S, Rolfe K, Julious SA. Investigating variability in patient response to treatment—a case study from a replicate cross-over study. *Stat Methods Med Res*. 2011;20(6):657–66.
17. Senn S. Mastering variation: variance components and personalised medicine. *Stat Med*. 2016;35:966–77.
18. Brown GL, Lean ME, Hankey CR. Reproducibility of 24-h post-exercise changes in energy intake in overweight and obese women using current methodology. *Br J Nutr*. 2012;108(2):191–4.
19. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381–95.
20. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res*. 1985;29(1):71–83.
21. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr*. 1978;40(3):497–504.
22. Siri WE. Body composition from fluid space and density. In: Brozek J, Hanschel A, editors. *Techniques for Measuring Body Composition*. Washington, DC: National Academy of Science; 1961. pp. 223–44.
23. Borg GA. Perceived exertion. A note on "history" and methods. *Med Sci Sports*. 1973;5(2):90–3.
24. Taylor HL, Buskirk E, Henschel A. Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol*. 1955;8(1):73–80.
25. Chandarana K, Drew ME, Emmanuel J, et al. Subject standardization, acclimatization, and sample processing affect gut hormone levels and appetite in humans. *Gastroenterology*. 2009;136(7):2115–26.
26. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol*. 1983;55(2):628–34.
27. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes*. 2000;24(1):38–48.

APPLIED SCIENCES

28. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*. 1974;37:247–8.

29. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988. pp. 22–5.

30. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3–13.

31. Laan DJ, Leidy HJ, Lim E, Campbell WW. Effects and reproducibility of aerobic and resistance exercise on appetite and energy intake in young, physically active adults. *Appl Physiol Nutr Metab*. 2010;35(6):842–7.

32. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M. Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol (1985)*. 2007;102(6):2165–71.

33. King JA, Miyashita M, Wasse LK, Stensel DJ. Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite*. 2010;54(3):492–8.

34. Deighton K, Barry R, Connon CE, Stensel DJ. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*. 2013;113(5):1147–56.

35. Sim AY, Wallman KE, Fairchild TJ, Guelfi KJ. High-intensity intermittent exercise attenuates ad-libitum energy intake. *Int J Obes*. 2014;38(3):417–22.

36. Martins C, Stensvold D, Finlayson G, et al. Effect of moderate- and high-intensity acute exercise on appetite in obese individuals. *Med Sci Sports Exerc*. 2015;47(1):40–8.

37. Blundell J, de Graaf C, Hulshof T, et al. Appetite control: methodological aspects of the evaluation of foods. *Obes Rev*. 2010;11(3):251–70.

38. Asmar A, Asmar M, Simonsen L, et al. Glucagon-like peptide-1 elicits vasodilation in adipose tissue and skeletal muscle in healthy men. *Physiol Rep*. 2017;5(3):e13073.

39. Edinburgh RM, Hengist A, Smith HA, et al. Prior exercise alters the difference between arterialised and venous glycaemia: implications for blood sampling procedures. *Br J Nutr*. 2017;117(10):1414–21.

40. Goodyear S, Arasaradnam RP, Quraishi N, Mottershead M, Nwokolo CU. Acylated and des acyl ghrelin in human portal and systemic circulations. *Mol Biol Rep*. 2010;37(8):3697–701.

41. Martin J, Smith J, Bastien M, et al. Comparison between arterial and venous sampling of circulating hormones, substrates and peptides in severe obesity. *Clin Invest Med*. 2011;34(2):E82–7.

42. Alajmi N, Deighton K, King JA, et al. Appetite and energy intake responses to acute energy deficits in females versus males. *Med Sci Sports Exerc*. 2016;48(3):412–20.

43. Douglas JA, King JA, Clayton DJ, et al. Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. *Int J Obes (Lond)*. 2017;41(12):1737–44.

44. Broom DR, Miyashita M, Wasse LK, et al. Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *J Endocrinol*. 2017;232(3):411–22.

APPLIED SCIENCES