Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: a randomized controlled trial

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Rosenkilde M, Reichkendler MH, Auerbach P, Toräng S, Gram AS, Ploug T, Holst JJ, Sjödin A, Stallknecht B. Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: a randomized controlled trial. J Appl Physiol 115: 1599–1609, 2013. First published September 19, 2013; doi:10.1152/japplphysiol.00680.2013.—Weight loss induced by endurance exercise is often disappointing; possibly due to an increase in energy intake mediated through greater appetite. The aim of this study was to evaluate fasting, postprandial, and postexercise appetite regulation after an intervention prescribing two amounts of endurance exercise. Sixty-four sedentary, overweight, healthy young men were randomized to control (CON), moderate-dose (MOD: ~30 min/day), or high-dose (HIGH: ~60 min/day) endurance exercise for 12 wk. Along with subjective appetite ratings, plasma ghrelin, glucagon, insulin, peptide YY3–36, glucose, free fatty acids, and glycerol were measured during fasting and in relation to a breakfast meal and an acute bout of exercise, both at baseline and at follow-up. Ad libitum lunch energy intake was evaluated 3 h after the breakfast meal. Despite different amounts of endurance exercise, the subjects lost similar amounts of fat mass (MOD: 4.2 ± 0.5 kg; HIGH: 3.7 ± 0.5 kg). Fasting and postprandial insulin decreased ~20% in both exercise groups (P < 0.03 vs. CON). Appetite measurements were not upregulated in the fasting and postprandial states. On the contrary, fasting and postprandial ratings of fullness and postprandial PYY3–36 increased in HIGH (P < 0.001 vs. CON). Ad libitum lunch energy intake remained unchanged over the course of the intervention. In both exercise groups, plasma ghrelin increased in relation to acute exercise after training. Thus neither moderate nor high doses of daily endurance exercise increased fasting and postprandial measures of appetite, but a high dose of exercise was associated with an increase in fasting and meal-related ratings of fullness and satiety.

Ad libitum EI, and discrepancies in appetite regulation between the two exercise groups could potentially explain why energy balance was equally negative.

Despite the complex regulation of food intake and energy homeostasis (10), under normal conditions, the result of an augmented appetite should be an increase in EI. Gastrointestinal hormones influence food intake and regulate both acute and long-term energy homeostasis (10). A range of hormones, including peptide YY (PYY)3–36 (1) and glucagon-like peptide 1 (GLP-1), provide negative feedback that inhibits appetite and food intake, whereas ghrelin is the only gastrointestinal hormone known to stimulate appetite (orexigenic properties) (11). In addition, hormones primarily thought to regulate glucose metabolism have also been implicated in energy homeostasis; e.g., insulin as a central regulator that decreases food intake (39). However, recently, it has also been hypothesized that glucagon, the glucose counterregulator, plays a role in energy homeostasis via inhibition of food intake (24).

In overweight and obese subjects, it has been reported that an increase in both the perceived and physiological drive to eat can act as a counterregulatory adaptation to weight loss induced by caloric restriction (14, 41). However, it is still controversial whether the potential negative energy balance induced by endurance exercise over time also stimulates appetite. More than 50 yr ago, it was suggested that EI was closely associated with, and regulated by, EE in lean subjects (15, 33). However, the increased EE associated with acute exercise is not linked to a concomitant increase in EI (23, 38). On the contrary, acute exercise sometimes suppresses appetite temporally (4, 45); this is known as “exercise anorexia”. In a series of supervised exercise intervention studies, Blundell and colleagues (27, 32) elegantly demonstrated that habitual exercise (12 wk, 5 days/week) exerted a dual effect on appetite among overweight and obese subjects (27, 32); the motivation to eat was higher in the fasting state, but postprandial satiety responses also improved. However, in these studies, only a single dose of exercise (EE: 500 kcal/day) was examined, and no control groups were included. Thus investigating the effects of different amounts of exercise (i.e., dose response) could provide a better understanding of appetite regulation in response to endurance exercise. Moreover, the exercise anorexia phenomenon observed after acute exercise is poorly described and could be adjusted after habituation to regular endurance exercise.

The purpose of the present study was to evaluate the effect of different doses of daily exercise on measurements of appetite regulation in previously sedentary, moderately overweight men. Randomizing the subjects into three groups, we investigated the effects of 12 wk of endurance exercise (correspond-

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Study population. Sixty-four healthy, moderately overweight [body mass index (BMI) 25–30 kg/m² and body fat percentage ≥25%], sedentary [maximal oxygen uptake (VO₂max) < 45 mL O₂/kg], young (20–40 yr) male subjects were recruited for participation in the study. A total of 135 subjects were screened; they were excluded for further participation if their blood pressure was ≥ 140/90 mmHg, fasting blood glucose was ≥ 6.1 mmol/L, or if they were dieting, already engaged in regular exercise, or regularly took medication. After receiving detailed information about the study, each participant signed informed consent. Withdrawal of consent or insufficient exercise compliance (less than 80% of the prescribed exercise) resulted in exclusion from the study. The study adhered to the principles in the Declaration of Helsinki, was approved by the Ethical Committee of the City of Copenhagen (H-4-2009-089), and was registered at clinicaltrials.gov (identifier: NCT01430143).

Study design. The data presented are part of a larger study on metabolic and cultural health in moderately overweight men [Project Four-IN-onE (FINE); fine.ku.dk/english] conducted between December 2009 and July 2011, and the overall design of the study, as well as data on body composition, free living energy intake, and resting metabolic rate have been described in detail elsewhere (37).

Briefly, subjects were block randomized (block size: 12) to one of three groups: a sedentary control group (CON: n = 18), a moderate-dose exercise group (MOD: n = 21), and a high-dose exercise group (HIGH: n = 22), using a manual lottery. The two exercise groups were instructed to engage in daily endurance exercise and to increase their exercise EE, intensity, and duration of each exercise session was individually prescribed and extensively monitored via frequent personal consultations with scientific staff (at least twice weekly). The exercise prescription was based on each subject's individual relationship between HR and VO₂, as determined by indirect calorimetry during an exercise test in the laboratory at baseline, and was recalibrated after the second, sixth, and tenth intervention week based on changes in VO₂max, HR, and body weight. Compliance was verified using HR monitors (RS400; Polar Electro, Kempele, Finland) that stored information about the subjects’ exercise EE, intensity, and duration.

All subjects underwent a preintervention testing regime and were subsequently randomized; they adhered to their randomization for 12 wk, wherein the last 2 wk were comprised of follow-up testing that included a testing regime identical to the prerandomization period.

Test days. We employed two metabolic challenges on two different test days: a single meal test and an acute exercise test. All subjects were instructed to register their food intake on the evening before the day of the test and to repeat the regime before follow-up testing, as well as to fast overnight before the test days. The two exercise groups were also directed to adhere to their prescribed exercise regimens on the day before follow-up testing. Body composition and VO₂max were measured after an overnight fast on a separate day and have been described elsewhere (37). Weight was measured on an electronic scale, height was measured using a stadiometer, and body composition was assessed using dual-energy X-ray absorptiometry (DPX-IQ X-ray bone densitometer 4.7c; Lunar, Madison, WI). VO₂max was measured using an electronically braked bicycle (Lode Excalibur, Groeningen, Netherlands) with incremental workloads, and VO₂max was accepted using objective criteria [leveling off in VO₂, respiratory-exchange ratio > 1.15, and maximal age-predicted HR (220-age)].

At the day of the single meal test, an intravenous catheter was inserted in a cubital vein, and, after a 15-min rest period, a baseline (0 min) blood sample was drawn (see Biochemical analyses). Subjective appetite sensations were subsequently rated using visual analog scales (VAS) for hunger, satiety, fullness, and prospective food intake. After this, a standardized breakfast was served (600 kcal: 63% carbohydrate, 24% fat, 14% protein), and blood samples and appetite ratings were obtained every 30 min for the next 180 min. Finally, subjects were served an ad libitum lunch meal. The lunch meal was a mixed hot pot pasta Bolognese. Subjects ate on their own in a quiet room without any external disturbances, and they were instructed to eat at a constant pace until they felt comfortably satiated and to subsequently rate their appetite using the VAS; this meal-test procedure to assess appetite has been validated elsewhere (18, 20). Immediately after the breakfast and lunch meals, additional VAS ratings were done to assess the subjective palatability of the meal, and the liking of the meal was tested using the same procedure.

At the day of the acute exercise test, an intravenous catheter was inserted in a dorsal hand vein, and subjects rested for 15 min. The hand was heated for 10 min at rest and for 5 min during exercise before a blood sample was drawn (see Biochemical analyses). After a baseline (0 min), blood sample was obtained, and appetite was subsequently rated using VAS for hunger, satiety, fullness, and prospective food intake. Subjects then commenced a 1-h exercise test, after which they rested in a comfortable bed for 1 h. The exercise test started incrementally, with the first workload (8 min) corresponding to 20% VO₂max; this was followed by five 3-min increments corresponding to 30–70% VO₂max until 21 min was reached. The remainder of the test proceeded at a workload that elicited 60% VO₂max. During these 2 h, blood samples were drawn every 30 min, and VAS was recorded at the 60-, 90-, and 120-min marks. At the 30- and 60-min marks, after the blood samples and VAS ratings were obtained, the subjects were offered a glass of water (250 ml) for their comfort.

Questionnaires. The 51-item Three-Factor Eating Questionnaire (29) was used to assess factors related to cognition and eating behaviors, specifically cognitive dietary restraint (intent to control food intake), disinhibition (overconsumption of food in response to cognitive or emotional cues), and susceptibility to hunger (food intake in response to feelings and perceptions of hunger). The questionnaire was administered on a separate day to limit its potential influence on subjects’ appetite assessments.

Biochemical analyses. Blood samples for the analysis of insulin, glucagon, and PYY3-36 were collected in a BD vacutainer (K3E Aprotinin 250 KIU; BD Biosciences, Franklin Lakes, NJ). Blood samples for the analysis of ghrelin, glucose, free fatty acids (FFA), and glycerol were stabilized with 1.5 mg EDTA per ml blood. The sample tubes were immediately centrifuged in a Hettich Rotina 35 R Centrifuge (Hettich, Tuttlingen, Germany) with a relative centrifugal force of 2,100 g for 10 min at 4°C, and plasma was subsequently stored at −80°C for later analysis. Concentrations of plasma insulin were determined using a commercially available ELISA kit (Dako, Glostrup, Denmark) and read on a Labsystems Multiskan MS (Thermo Electron, Vantaa, Finland). Total ghrelin and PYY3-36 were measured using radioimmunoassay with commercially available kits (Linco Research, St. Charles, MO). After the extraction of plasma, glucagon was measured with 70% ethanol (vol/vol, final concentration). The antibody utilized (code no. 4305) is directed toward the COOH terminus of the glucagon molecule; therefore, it mainly measures glucagon of pancreatic origin (22). The standards were human glucagon, and the tracer was monoidinated human glucagon (both gifts from Novo Nordisk, Bagsværd, Denmark). The sensitivity and
detection limit was below 1 pmol/l, the intra-assay coefficient of
variation was below 6% at 20–30 pmol/l, and the recovery of the
standard (added to the plasma before extraction) was about 100%
when corrected for losses inherent in the plasma-extraction procedure.
Plasma glucose, FFA, and glycerol were each analyzed using glu2
(Roche Diagnostics; Mannheim, Germany), NEFA-HR (2) (Wako
Chemicals, Neuss, Germany), and kit no. 10148270035 (Roche Di-
agnostics; Hvidovre, Denmark), respectively, on the 111-Cobas
(Roche Diagnostics, Germany).

Calculations and statistical analysis. Fasting appetite scores (as
assessed by VAS) as well as plasma hormones and metabolites were
averaged from both the meal-test and exercise-test days. The total area
under the curve (t-AUC) for VAS, plasma hormones, and metabolites
were calculated using the trapezoidal rule. Furthermore, average hor-
mones and metabolite concentrations were calculated both during
exercise (30 and 60 min) and during recovery (90 and 120 min). The
EE measured during the exercise test was calculated using the Weir
equation (46).

The prespecified outcomes of the study were registered at
clinicaltrials.org (NCT01430143). Peripheral insulin sensitivity was
the primary outcome and has been published elsewhere (36), whereas
changes in body composition and appetite were secondary outcomes
and evaluated in the present study. This study was an efficacy study, and the
results are presented as per-protocol analyses. Mean data for the individ-
ual groups at baseline and follow-up are tabulated as means ± SD unless
otherwise noted. Differences between the groups were assessed using
analysis of covariance, with individual preintervention values and
group assignment as covariates. The differences in follow-up values
among the randomization groups are presented as adjusted least-
square means with 95% confidence limits, and all pairwise compar-
sions were adjusted using the Tukey procedure. Differences within
the groups were tested using simple unadjusted t-tests and are tabulated as
means with 95% confidence limits. A mixed model with systematic
effects of group (CON as reference) and visit (pretest vs. follow-up
test) with random levels for individuals and repeated measurements
over testing time points (time 0–180 min) and autoregressive error
effects of group (CON as reference) and visit (pretest vs. follow-up
visit, group

RESULTS

Subject characteristics and exercise intervention. Of the 64
subjects deemed eligible for participation in the study, three
subjects withdrew their informed consent during the preinter-
vention testing, leaving 61 volunteers eligible for randomiza-
tion. Fifty-three subjects completed the intervention (CON:
n = 17; MOD: n = 18; HIGH n = 18); reasons for withdrawal were lack of motivation (CON: n = 1; HIGH: n = 3), low exercise compliance (MOD: n = 3), or the reemergence
of an earlier injury (HIGH: n = 1). Due to technical problems
with the online equipment at the exercise test, one subject in
HIGH and one subject in CON were not tested at baseline, and
one subject in CON was not tested at follow-up due to time
constraints. Thus we did not obtain data for these subjects at
these test days.

Overall, the subjects’ adherence to exercise was excellent,
with MOD having marginally better compliance to the pre-
scribed amount of exercise (in kcal) than HIGH, as measured
until the meal test (MOD: 99 ± 1% vs. HIGH: 96 ± 1%;
difference between MOD and HIGH: 3.7% CI: −7.5: −0.5%,
P = 0.047). The average exercise intensity was not different
(MOD: 66 ± 1% VO2max vs. HIGH: 67 ± 1% VO2max, P = 0.62), but, as intended by the study design, HIGH expended
more energy on average during their prescribed exercise
(MOD: 338 ± 8 kcal vs. HIGH: 649 ± 10 kcal, P < 0.001)
and, therefore, a greater amount of time per exercise session
(MOD: 30 ± 2 min vs. HIGH: 55 ± 2 min, P < 0.001).

At follow-up, both groups had achieved similar reductions
in body weight and fat mass, regardless of the amount
of exercise; however, fat-free mass tended to increase in
HIGH compared with CON (P = 0.06) (see Table 1) (37). In
both groups, VO2max increased similarly in response to exercise
(see Table 1) (37).

Fasting measurements. After the intervention, and regard-
less of the amount of exercise, fasting plasma concentrations of
insulin had reduced by 23% (CI: 3, 43%) in MOD and 22%
(CI: 2, 42%) in HIGH compared with CON; in addition, the
concentration of PYY3–36 tended to increase in HIGH compared
with CON (9.5 pg/ml CI: −0.7, 19.7, P = 0.07) (Table 2). In
the fasting state, subjects’ ratings of fullness and satiety increased
in HIGH compared with both MOD and CON (Table 2).

Table 1. Subject characteristics at baseline and in the end of the intervention

<table>
<thead>
<tr>
<th>CON (n = 17)</th>
<th>MOD (n = 18)</th>
<th>HIGH (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest</td>
<td>Follow-up</td>
<td>Pretest</td>
</tr>
<tr>
<td>Age, yr</td>
<td>31 (6)</td>
<td>30 (7)</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>92.8 (8.5)</td>
<td>92.9 (8.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.0 (2.3)</td>
<td>28.1 (2.4)</td>
</tr>
<tr>
<td>Fat Mass, kg</td>
<td>29.0 (6.0)</td>
<td>28.9 (5.8)</td>
</tr>
<tr>
<td>Fat-free Mass, kg</td>
<td>63.9 (5.8)</td>
<td>64.1 (6.1)</td>
</tr>
<tr>
<td>VO2max, ml/kg/min</td>
<td>35.9 (5.0)</td>
<td>37.1 (3.5)</td>
</tr>
</tbody>
</table>
| Descriptive data are means (SD), and change scores are least square means (95% confidence interval). Fat and fat-free mass was determined by Dual X-ray Absorptiometry scanning. Maximal oxygen uptake was measured during an incremental bicycle test. *Significant within group. BMI, body mass index; CON, control; MOD, moderate-exercise group, 30 min/day; HIGH, high-exercise group, 60 min/day; VO2max, maximal oxygen uptake.
Table 2. Fasting plasma hormones and metabolites and perceived appetite sensations

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 17)</th>
<th>MOD (n = 18)</th>
<th>HIGH (n = 18)</th>
<th>P vs. CON</th>
<th>P vs. MOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Follow-up</td>
<td>Pretest</td>
<td>Follow-up</td>
<td>Δ Relative to CON</td>
</tr>
<tr>
<td><strong>Plasma Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghrelin, pg/mla</td>
<td>674 (136)</td>
<td>704 (122)</td>
<td>698 (209)</td>
<td>714 (208)</td>
<td>−13 (−97:72)</td>
</tr>
<tr>
<td>Insulin, pmol/lb</td>
<td>50 (15)</td>
<td>52 (18)</td>
<td>52 (14)</td>
<td>41 (12)*</td>
<td>−12 (−22:−2)</td>
</tr>
<tr>
<td>Glucagon, pmol/lb</td>
<td>9.3 (3.4)</td>
<td>9.8 (2.7)</td>
<td>10.5 (3.5)</td>
<td>10.4 (3.5)</td>
<td>0.2 (−2:3.27)</td>
</tr>
<tr>
<td>PYY3–36, pg/ml</td>
<td>47 (23)</td>
<td>48 (19)</td>
<td>18 (18)</td>
<td>1.0 (−9:11)</td>
<td>0.97</td>
</tr>
<tr>
<td>Glucose, mmol/lc</td>
<td>5.5 (0.1)</td>
<td>5.6 (0.2)</td>
<td>5.4 (0.2)</td>
<td>5.3 (0.2)</td>
<td>−0.3 (−0.5:−0.3)</td>
</tr>
<tr>
<td>Glyceral, µmol/l</td>
<td>406 (85)</td>
<td>415 (87)</td>
<td>428 (110)</td>
<td>469 (124)</td>
<td>70 (−15:155)</td>
</tr>
<tr>
<td><strong>Appetite Sensations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger, mm</td>
<td>54 (15)</td>
<td>61 (16)</td>
<td>51 (17)</td>
<td>60 (16)</td>
<td>0 (−12:13)</td>
</tr>
<tr>
<td>Satiety, mm</td>
<td>31 (12)</td>
<td>27 (13)</td>
<td>31 (12)</td>
<td>28 (16)</td>
<td>1 (−9:10)</td>
</tr>
<tr>
<td>Fullness, mm</td>
<td>28 (13)</td>
<td>21 (11)</td>
<td>25 (14)</td>
<td>20 (12)</td>
<td>0 (−10:10)</td>
</tr>
<tr>
<td>Prospective Food</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Consumption, mm</td>
<td>56 (13)</td>
<td>63 (13)</td>
<td>61 (13)</td>
<td>65 (12)</td>
<td>0 (−9:9)</td>
</tr>
<tr>
<td><strong>Three-Factor Eating Questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Restraint</td>
<td>3.9 (2.0)</td>
<td>4.4 (2.6)</td>
<td>6.8 (2.3)</td>
<td>4.1 (2.6)</td>
<td>0.2 (−2:1.25)</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>7.3 (2.6)</td>
<td>6.5 (2.4)</td>
<td>6.7 (2.4)</td>
<td>6.1 (2.2)</td>
<td>−0.1 (−1:1.86)</td>
</tr>
<tr>
<td>Susceptibility to Hunger</td>
<td>6.6 (2.4)</td>
<td>6.8 (2.4)</td>
<td>5.0 (3.4)</td>
<td>4.1 (2.6)</td>
<td>−1.6 (−3:2.0)</td>
</tr>
</tbody>
</table>

Descriptive data are means (SD), and change scores are least square means (95% confidence interval). Values are determined using t-radio immune assay, *ELISA, †Cobas, and ++visual analog scales. *Significant within group, †significant versus MOD, PYY, peptide YY; FFA, free fatty acids.

Compared with CON, there was a decrease in fasting concentrations of glucose and a tendency toward less susceptibility to hunger (P = 0.06) in MOD (Table 2).

**Meal test.** In relation to the breakfast meal, the reduced fasting concentrations of insulin in the two exercise groups persisted, as illustrated by a decreased t-AUC for both MOD (P = 0.015) and HIGH (P = 0.020). The analysis of repeated measurements demonstrated a significant group × visit interaction, which showed that insulin concentrations were 20% lower for MOD (estimate: −0.085, CI: −0.014; −0.003, P = 0.004) and 22% lower for HIGH (estimate: −0.088, CI: −0.014; −0.030, P = 0.003) at all time points compared with CON (Fig. 1). In relation to the breakfast meal, the tendency for an increased fasting concentration of PYY3–36 in HIGH was manifested as an increase in PYY3–36 concentration by 20% (group × visit interaction) in HIGH compared with CON (estimate: 0.078, CI: 0.053:0.131, P = 0.003) (Fig. 1). Likewise, increased fasting ratings of fullness in relation to the meal also persisted in HIGH compared with CON (estimate: 0.163, CI: 0.081:0.245, P < 0.001) (Fig. 2). There was no group × visit interaction for hunger (P > 0.75), but ratings of hunger were 34–50% lower 30 and 60 min after the breakfast in both MOD and HIGH compared with CON (P < 0.05 for all comparisons) (Fig. 2). This was in accordance with a similar reduction (27–33%) in ratings of prospective food intake in HIGH at identical time points (P < 0.012) compared with CON (Fig. 2). In relation to the standardized breakfast, there was no difference in t-AUC for plasma glucose, FFA, or glyceral among the three groups at follow-up (P > 0.33 for all), but there was a general decrease in glyceral response in HIGH (P = 0.03 vs. CON for time × treatment interaction) (Fig. 3).

After the intervention, there was no difference in ad libitum EI within MOD or HIGH or compared with CON (CON vs. MOD: −102 kcal, CI: −244:40, P = 0.20; CON vs. HIGH: −96 kcal, CI: −236:44, P = 0.23) (Fig. 4). The subsequent palatability ratings of the ad libitum meal indicated that the lunch meal was liked equally well (P > 0.93 for all groups), and the satisfaction with the meal was also unchanged within each group (P > 0.20 for all). However, as shown in Fig. 4, three subjects displayed a remarkable change in ad libitum EI from preintervention to follow-up (MOD: from 145 to 750 kcal, and from 120 to 590 kcal; CON: from 1,480 to 615 kcal). These subjects did not display any adverse subjective appetite ratings immediately before the meal, nor was their satisfaction with the meal apparently different. After excluding these three subjects, a tendency toward a decrease in EI was observed in MOD compared with CON at follow-up (−115 kcal, CI: −244:13; P = 0.08).

**Exercise test.** Due to increases in V˙O2max, the absolute workload eliciting 60% of V˙O2max increased in HIGH compared with CON (P = 0.049) and from preexercise to follow-up in both MOD (P = 0.020) and HIGH (P < 0.001). Within both groups, this increase resulted in increased EE (in kcal) (Table 3).

In response to the intense bout of exercise, levels of hormones (insulin, glucagon, ghrelin, and PYY3–36) remained unchanged in both MOD and HIGH compared with CON; this is summarized by t-AUC (Fig. 1). However, within both exercise groups, the t-AUC for ghrelin increased (MOD: P = 0.036; HIGH: P = 0.027) after the exercise intervention. The t-AUC for glucagon increased within CON (P = 0.005), and the t-AUC for insulin decreased within MOD at follow-up (P = 0.015) (Fig. 3). Also at follow-up, t-AUC for glyceral was increased in HIGH in relation to the intense bout of exercise (P = 0.003). During exercise (at the 30- and 60-min marks), the plasma glyceral concentration also increased in both
MOD (67 μmol/l, CI: 8:126, P = 0.028) and HIGH (68 μmol/l, CI: 27:109, P = 0.003) (Fig. 3). Also during exercise (at the 30- and 60-min marks), the average plasma FFA concentrations in HIGH increased from pretest to follow-up (194 μmol/l, CI: 52:334; P = 0.01) (Fig. 3). Consistent with this, the respiratory-exchange ratio decreased within both exercise groups (MOD: -0.02, CI: -0.05: -0.002, P = 0.04; HIGH: -0.03, CI: -0.04: -0.02, P < 0.001; Table 3). As measured by t-AUC, the overall rating of fullness increased within HIGH (P = 0.033) in relation to the exercise test (Fig. 2).

Correlations. From pretest to follow-up, changes in fasting concentrations of plasma PYY3–36 correlated positively with
changes in fasting fullness ($R = 0.29$, $P = 0.03$, $n = 53$). Also, in relation to the meal test, changes in plasma PYY3–36 t-AUC correlated positively with changes in t-AUC for fullness ($R = 0.31$, $P = 0.03$, $n = 53$) and satiety ($R = 0.32$, $P = 0.02$, $n = 53$). Moreover, there were negative correlations with changes in subjective ratings of hunger ($R = -0.27$, $P = 0.05$, $n = 53$) and prospective food intake ($R = -0.47$, $P < 0.001$, $n = 53$), but this was not found with the remaining hormones measured. Changes in ad libitum EI during the lunch meal did not correlate with changes in fasting and t-AUCs for subjective appetite ratings ($P > 0.19$) or changes in fasting concentrations or t-AUCs for any of the measured hormones ($P > 0.41$).
DISCUSSION

In the context of a 12-wk randomized, controlled trial that prescribed two different amounts of daily endurance exercise (300 or 600 kcal/day), we investigated subjects’ appetite regulation in the fasting state and in response to two inverse metabolic stimuli: a single meal test and an acute bout of exercise. To our knowledge, this is the first study to investigate the effects of different amounts of endurance exercise on the regulation of appetite. Our main finding was that, after engaging in a rigorous exercise intervention, measurements of appe-
The potential homeostatic mechanisms that oppose a negative energy balance induced by chronic caloric restriction are increased postprandial concentrations of ghrelin (12, 41) and/or a blunted PYY3–36 response (34) associated with increased motivation to eat (i.e., hunger sensation) (41). This counterregulatory response might differ when the energy deficits are produced by exercise. In response to acute energy deficits, both hormonal and subjective appetite markers, as well as subsequent ad libitum EI, increased with acute food restriction but were absent with acute exercise when the energy deficit was the same in the two conditions (25). After subjects’ engagement in the 12-wk exercise intervention, we did not detect any signs of an increased homeostatic drive to eat (e.g., ratings of hunger) in the fasting condition; this is in contrast to earlier studies that did not include control groups (27, 32). Instead, we noted that opposite signals, perceived satiety, and fullness, as well as plasma PYY3–36 (only a statistical tendency), increased in HIGH. When MOD and HIGH were combined, ratings of fasting hunger increased (7 mm CI: 1:13, \(P = 0.03\)), but this apparent increase disappeared compared with CON, which also tended to increase their fasting hunger ratings (7 mm, CI: \(-1:15\), \(P = 0.07\)). This order effect on fasting hunger sensations possibly arises due to changed expectations from test conditions (i.e., pretest vs. posttest). Although the use of VAS scales for subjective appetite sensations has been validated using crossover designs (18), their use in longitudinal designs has not. This emphasizes the importance of the controlled nature of the present longitudinal study. In addition, an increase in fasting ghrelin has previously been implicated as a compensatory response to exercise-induced weight loss in women (19, 31) but not in men (35), as the present study confirms.

The immediate postprandial satiety response (satiation, i.e., the cessation of eating) clearly seems to improve after habituation to exercise, as assessed by both subjective ratings (21, 27) and hormonal mediators (32). In the present study, we found that immediate postprandial (30–60 min marks) hunger ratings decreased after 3 mo of endurance exercise, which confirms an exercise-induced improvement in satiation. Thus our findings support, in terms of hunger ratings, that exercise does decrease immediate postprandial hunger ratings but not fasting ratings. The absence of a postprandial increase in plasma ghrelin after the exercise intervention is also consistent with earlier findings from similar interventions (21, 32). After the intervention, the satiating effect of the standardized breakfast was more pronounced in HIGH compared with CON; fullness, satiety, and plasma PYY3–36 also increased in HIGH. Regardless of the two exercise groups’ increased satiation immediately after the breakfast, their ad libitum EI during the lunch meal did not change as a result of the intervention.

From the results of the present study, we have reported that energy balance (calculated from changes measured in body composition) was substantially more negative (\(-80\%\)) in MOD than could be explained by the EE associated with the group’s actual amount of exercise (37); possibly, this is due to a decrease in EI. However, within HIGH, the group’s energy balance was less negative (\(-20\%\) or 120 kcal/day) than expected, based on their exercise program; as measured by weighed food records (37), subjects’ habitual EI increased numerically (130 kcal/day). This appears to contradict the present findings of increased fasting fullness and satiating...
response to a prepared meal, as found in this group. Measures of appetite were performed in the morning and within a limited time frame (3 h postprandial and 1 h after exercise) and thus show a short-term effect, which possibly does not persist across 24 h. If this was the case, a greater loss of body weight and fat would be expected in HIGH. We acknowledge that changes in food intake and/or appetite at other points of time than those examined in the present study might be involved, which could explain this. In addition, we only addressed homeostatic aspects of appetite regulation, whereas hedonic aspects of appetite control and energy/food intake (i.e., eating for pleasure) were not investigated; these factors may also affect exercise-induced changes in body composition (17).

At follow-up, both exercise groups showed a decrease in plasma insulin, which we observed in the fasting state as well as in relation to the meal and acute exercise. We ascribe this to an exercise-induced increase in both hepatic and peripheral insulin sensitivity, as previously described in relation to the same subjects (36). Plasma insulin oscillates (e.g., in response to feeding), but insulin also acts as an adiposity signal via the regulation by the central nervous system of food intake (39). In addition to its well-known function to restore concentrations of blood glucose during hypoglycemia, glucagon has also been shown to have satiating properties in humans (24). We did not find that endurance exercise had an effect on fasting plasma glucagon, either in relation to a meal or acute exercise. Recently, however, it has been proposed that glucagon is part of the incrementing postprandial gastroenteropancreatic response to protein-induced satiety, which increases with meal protein content (2).

A novel finding of the present study is that, in response to the acute bout of exercise at follow-up, plasma ghrelin increased in both exercise groups, but not in CON. It has been consistently shown that an acute bout of vigorous, high-intensity exercise induces a reduction in both the subjective and hormonal mediators of appetite (5, 28, 29, 40). Studies have demonstrated that total ghrelin decreases (43) or increases (16) with acute exercise, whereas the acylated ghrelin component (orexigenic) is suppressed after an acute bout of exercise (7, 8). A major limitation of the present study was that acylated ghrelin was not measured. However, after a comparable bout of exercise (1 h at 70% VO\textsubscript{2max}), both total and acylated ghrelin increased in endurance-trained female runners but not in untrained female walkers (30). Conversely, Ueda et al. (44) recently reported that, after 12 wk of endurance exercise (3 days/wk), an increase in GLP-1 and total PYY but not ghrelin among middle-aged Japanese women in response to acute exercise (44) was observed. Discrepancies in age, amounts of exercise, ethnicity, and sex could account for the different outcomes of our study.

The current study benefits from a robust design (a randomized, controlled, dose-response trial), and the methodology used to assess appetite regulation is considered to have a high validity (3). However, it would have been particularly advantageous to study a larger sample size, as the individual response measured in weight/fat loss, and thereby the compensatory response to endurance exercise, is highly variable (26). Other limitations are that the duration of this study was perhaps too short to identify an increase in measurements of appetite, which are likely to occur at some time point to preserve energy stores and that appetite was assessed by limited methodology (VAS and a few hormones known to affect appetite) in a limited time frame (a few hours during a 12-wk exercise intervention). Also, a valuable addition would have been to determine ad libitum EI subsequent to the acute exercise bout. The study was an efficacy study, and we applied a per-protocol statistical analysis restricted to completers. This procedure somewhat limits the practical implications of our findings.

In conclusion, overweight, previously sedentary men’s participation in 12 wk of 30 or 60 min of daily endurance exercise did not result in signs of increased appetite despite the presence of a negative energy balance. Instead, fasting and postprandial perceptions of satiety and fullness, as well as concentrations of PYY\textsubscript{3–36}, increased after a high dose of daily endurance exercise. This short-term effect on markers of appetite does not elude why energy balance was equally negative between MOD and HIGH. Moreover, in response to an acute bout of exercise, plasma ghrelin increased from baseline to follow-up, regardless of the dose of endurance exercise; this indicates enhanced sensitivity to an energy deficit after endurance exercise.

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REFERENCES


