2016

# Low resting metabolic rate in exercise-associated amenorrhea is not due to a reduced proportion of highly active metabolic tissue compartments

Karsten Koehler
*University of Nebraska - Lincoln*, kkoehler3@unl.edu

Nancy I. Williams
*The Pennsylvania State University*, niw1@psu.edu

Rebecca J. Mallinson
*The Pennsylvania State University*, mallinsonrj@gmail.com

Emily A. Southmayd
*The Pennsylvania State University*, emily.southmayd@gmail.com

Heather C.M. Allaway
*The Pennsylvania State University*, hca111@psu.edu

***See next page for additional authors***

**Citation:** Koehler K, Williams NI, Mallinson RJ, Southmayd EA, Allaway HC, De Souza MJ. Low resting metabolic rate in exercise-associated amenorrhea is not due to a reduced proportion of highly active metabolic tissue compartments. *Am J Physiol Endocrinol Metab* 311: E480–E487, 2016. doi:10.1152/ajpendo.00110.2016.




Submitted 21 March 2016; accepted 1 July 2016; published July 5, 2016.

# Low resting metabolic rate in exercise-associated amenorrhea is not due to a reduced proportion of highly active metabolic tissue compartments

Karsten Koehler, Nancy I. Williams, Rebecca J. Mallinson, Emily A. Southmayd, Heather C. M. Allaway, and Mary Jane De Souza

Department of Kinesiology, Pennsylvania State University, University Park, Pennsylvania, USA

*Corresponding author* — M. J. De Souza, Dept. of Kinesiology, Pennsylvania State University, University Park, PA 16802; email mjd34@psu.edu
*Present address of K. Koehler* — Department of Nutrition and Health Sciences, University of Nebraska–Lincoln, Lincoln, NE.

**Abstract**

Exercising women with menstrual disturbances frequently display a low resting metabolic rate (RMR) when RMR is expressed relative to body size or lean mass. However, normalizing RMR for body size or lean mass does not account for potential differences in the size of tissue compartments with varying metabolic activities. To explore whether the apparent RMR suppression in women with exercise-associated amenorrhea is a consequence of a lower proportion of highly active metabolic tissue compartments or the result of metabolic adaptations related to energy conservation at the tissue level, RMR and metabolic tissue compartments were compared among exercising women with amenorrhea (AMEN; $n$ = 42) and exercising women with eumenorrheic, ovulatory menstrual cycles (OV; $n$ = 37). RMR was measured using indirect calorimetry and predicted from the size of metabolic tissue compartments as measured by dual-energy X-ray absorptiometry (DEXA). Measured RMR was lower than DEXA-predicted RMR in AMEN (1,215 ± 31 vs. 1,327 ± 18 kcal/day, $P$ < 0.001) but not in OV (1,284 ± 24 vs. 1,252 ± 17, $P$ < 0.16), resulting in a lower ratio of measured to DEXA-predicted RMR in AMEN (91 ± 2%) vs. OV (103 ± 2%, $P$ < 0.001). AMEN displayed proportionally more residual mass ($P$ < 0.001) and less adipose tissue ($P$ = 0.003) compared with OV. A lower ratio of measured to DXA-predicted RMR was associated with lower serum total triiodothyronine (ρ = 0.38, $P$ < 0.001) and leptin (ρ = 0.32, $P$ = 0.004). Our findings suggest that RMR suppression in this population is not the result of a reduced size of highly active metabolic tissue compartments but is due to metabolic and endocrine adaptations at the tissue level that are indicative of energy conservation.

**Keywords:** energy deficiency, metabolic adaptation, dual-energy X-ray absorptiometry, lean body mass, exercise-associated menstrual disturbances

Chronic energy deficiency results in a variety of metabolic adaptations that serve to conserve energy for essential processes such as cellular maintenance, thermoregulation, and locomotion (45). Energy-demanding processes not necessary for survival, including reproduction, may be surrendered in an environment of energy deficiency (45). Among women of reproductive age, severe energy deficiency is associated with impaired reproductive function, including functional hypothalamic amenorrhea (AMEN) (39). As a result of these energy-conserving adaptations, resting metabolic rate (RMR) is suppressed in chronically energy-deficient women, as observed in anorexia nervosa patients who exhibit RMRs that are 20–40% lower than expected (29, 38, 40). Likewise, lower than expected RMRs have also been reported in women with exercise-associated menstrual disturbances (EAMD) (10, 15, 21), a condition caused by insufficient dietary energy intake relative to the energy expenditure of exercise (13). Because women with EAMD frequently display disrupted profiles of leptin, triiodothyronine, insulin, and other peptides involved in energy homeostasis, their lower than expected RMRs are understood as evidence of energy conservation (10, 12, 42).

However, because RMR varies considerably among individuals, it is difficult to quantify the full extent of RMR suppression in response to chronic energy deficiency. Previous investigators have addressed this issue by normalizing RMR for lean mass (10, 15, 21, 32) or by comparing measured RMR with predicted RMR, which is derived from prediction equations that utilize anthropometric characteristics (10, 15, 21, 29, 38, 40). However, these approaches may not be adequately sensitive to reflect the true magnitude of RMR suppression (42). RMR is a product of the metabolic activity of various tissues, and metabolic rates vary considerably among these compartments (16). For example, the brain and vital organs are characterized by high energy consumption, whereas skeletal muscle, adipose tissue, and bone require considerably less energy at rest (16), and changes in the size or proportion of these compartments will result in characteristic differences in whole body RMR.

Therefore, a more in-depth analysis of RMR and the size of metabolically active tissues is required to assess whether the apparent RMR suppression in chronically energy-deficient women is simply a result of a reduced size of energetically expensive tissues or is in fact due to a reduction in metabolic rate at the tissue level. The size of metabolically active tissues can be estimated using advanced imaging techniques such as whole body dual-energy X-ray absorptiometry (DEXA), a technique widely used for body composition and bone mineral density assessment (25). With this knowledge, whole body RMR can be predicted using tissue-specific metabolic rates (18, 25, 46). Since this approach accounts for differences in the size of metabolically active tissue compartments, a reduced ratio of measured RMR to RMR predicted from the size of metabolically active tissues could be a more sensitive indicator of energy conservation when compared with RMR predicted from anthropometric characteristics or lean mass. To our knowledge, the comparison of measured and DEXA-predicted RMR has been used only to document RMR suppression in anorexia nervosa patients (30). No investigator to date has used whole body DEXA to examine the underlying mechanisms of RMR suppression in women with EAMD, a population that is of interest due to *1*) the frequency of menstrual disturbances, which can be as high as 60% among exercising women (20), and *2*) the association of

menstrual disturbances with poor skeletal, metabolic, and cardiovascular health (13).

The purpose of our study was to explore whether the lower than expected RMR in women with EAMD is explained by a lower proportion of tissues with high metabolic activity or whether it is due to metabolic adaptations indicative of energy conservation. To this end, we sought to compare RMR, as measured by indirect calorimetry, with RMR derived from whole body tissue analysis in women at opposite ends of the EAMD spectrum in 1) exercising women with functional hypothalamic AMEN and 2) a control group of exercising women with ovulatory menstrual cycles (OV). We hypothesized that measured RMR would be significantly lower than DEXA-predicted RMR only in the AMEN group, whereas measured and DEXA-predicted RMR would not be different in the OV group. We further hypothesized that when compared with ovulatory exercising women, AMEN women would not demonstrate a lower proportion of energetically expensive tissues such as brain, skeletal muscle, or residual mass but would demonstrate endocrine markers indicative of metabolic adaptations and energy conservation such as reduced triiodothyronine and leptin concentrations.

## Methods

*Study design.* The current study is a cross-sectional analysis comparing exercising women with amenorrhea (AMEN) with exercising women with ovulatory menstrual cycles (OV), who served as controls. The study merges data from two data sets to include data from a cross-sectional study on bone health among women of varying menstrual status (15) and the baseline period of a randomized controlled trial designed to determine the effects of a dietary intervention of increased caloric intake on bone health and menstrual status in women with EAMD (33, 35), with initial enrollments of $n$ = 54 and $n$ = 233, respectively. Both studies were approved by the Institutional Review Boards at the Pennsylvania State University and/or the University of Toronto. Both studies were conducted by the same laboratory, and procedures were standardized between protocols. The present analysis combines indirect calorimetry assessment of RMR and metabolic tissue compartment sizes derived from DEXA. Participants completed a monitoring period of 28 days (for AMEN women) or one menstrual cycle (for OV women), during which menstrual status was confirmed. All measurements occurred during or immediately before or after the monitoring period, and participants were instructed to remain weight stable and maintain their habitual exercise and dietary regimen during this time. The number and order of visits varied based on design of the initial studies and the participants' availability, but typically, all measurements were completed within three to five visits.

*Participants.* Participants were recruited via fliers, classroom announcements, and newspaper and Internet advertisements. In the current analysis, participants were included if they met the following criteria: *1*) age 18–35 yr, *2*) body mass index between 18 and 30 kg/m$^2$, *3*) ≥2 h of purposeful moderate- to high-intensity exercise/wk, *4*) nonsmoking, and *5*) self-reported and laboratory evidence of AMEN or regular ovulatory menstrual cycles, as described below. Women were excluded if they had any health condition or were taking medications that would affect metabolism or had a current clinical diagnosis of an eating or psychiatric disorder. Women with elevated serum concentrations of prolactin, thyroid-stimulating hormone, and/or androgens were also excluded (1). To minimize the impact of menstrual cyclicity on RMR (2, 37, 47), we further excluded participants in whom RMR data was not collected during the 1st wk (*days 2–6*) of the follicular phase.

*Classification of menstrual status.* Classification of menstrual status was based on self-reported menstrual history and confirmed by measurement of daily urinary reproductive hormone metabolites, estrone-1-glucuronide (E1G), pregnanediol glucuronide (PdG), and luteinizing hormone (LH) for one complete menstrual cycle in OV or a 28-day monitoring period in AMEN. Urinary measurement of E1G, PdG, and LH was conducted as published previously (14, 34). In brief, in-house competitive immunoassays were used to determine urinary E1G and PdG, and urinary LH was determined using an immunoradiometric assay (Siemens Healthcare Diagnostics, Deerfield, IL). Concentrations of these urinary metabolites were corrected for specific gravity to adjust for hydration. Amenorrhea was defined as self-reported absence of menses for ≥90 days and was confirmed by chronically suppressed concentrations of E1G and PdG during the monitoring period (14). Women were considered ovulatory if they reported at least nine menstrual periods in the past year and demonstrated a menstrual cycle length between 26 and 35 days, an E1G peak concentration >35 ng/ml, an LH surge concentration >25 mIU/ml that occurred on or after the day of the E1G peak, and a PdG peak concentration >5 μg/ml during the luteal phase (14, 15). Ovulatory women with luteal phase defects, as defined by a luteal phase <10 days in length (short luteal phase) or a peak luteal PdG concentration between 2.5 and 5 μg/ml (inadequate luteal phase), were included in the OV group (14). However, eumenorrheic women who presented with an anovulatory cycle, as defined by peak luteal PdG <2.5 μg/ml (14), were excluded.

*Anthropometrics, health, and fitness.* After participants gave informed consent, their height and body mass were measured and their menstrual history, physical activity, and medical and psychological health assessed using lab-specific questionnaires, which asked for past and current menstrual status, past physical activity and current habitual exercise, overall health, injuries, use of medication, and previous diagnosis of a clinical eating disorder. To detect signs of an eating disorder, participants completed the Eating Disorder Inventory-2 (19) and subsequently met with a psychologist who completed a semistructured interview to exclude this diagnosis. During a separate visit, most participants (81%) underwent a progressive treadmill test for peak oxygen uptake ($\dot{V}_{O_2}$ peak). Following a brief warmup, participants started running at a comfortable pace and a 0% incline. The incline was increased gradually by 2% every 2 min for the first 6 min and then by 1% every minute thereafter until volitional exhaustion was reached. Throughout the test, oxygen uptake was measured using an on-line MedGraphics (St. Paul, MN) Modular $\dot{V}_{O_2}$ System or Sensor- Medics (Yorba Linda, CA) $V_{max}$ metabolic cart, as described previously (42).

*Resting metabolic rate.* RMR was assessed via indirect calorimetry using a ventilated hood ($V_{max}$; CareFusion, Yorba Linda, CA), as described elsewhere (8). In short, testing occurred between 0600 and 1000 following an overnight fast and abstention from alcohol, caffeine, and exercise for ≥24 h. After subjects rested in a supine position for 30–45 min, a ventilated hood was placed over their head, and respiratory gas exchange was measured for ≥30 min. RMR was calculated from steady-state oxygen uptake and carbon dioxide production (49). RMR measurements were conducted either during or immediately following the menstrual status monitoring period. To minimize the impact of menstrual cyclicity on RMR (2, 37, 47), only RMR data collected during the early follicular phase, defined as the 1st wk after the onset of menses, were included in the analysis.

*Whole body dual energy X-ray absorptiometry.* Whole body DEXA scans were performed by a certified technician. In the majority of participants (71%), DEXA measurements were conducted prior to the start of the monitoring period. In the remaining participants (29%), we included DEXA data that were collected within 1 wk of the RMR. Participants were scanned on a GE Lunar (Madison, WI) Prodigy DEXA scanner (enCORE 2002 software, version 6.50.069) ($n$ = 49), a GE Lunar iDEXA scanner ($n$ = 24) (enCORE 2008 software version 12.10.113), or a Hologic QDR4500W DEXA scanner ($n$ = 6) (Hologic, Bedford, MA). Cross-calibration studies consistent with the guidelines of the International Society of Clinical Densitometry were performed to remove

system bias. For cross-calibration between the Lunar Prodigy and iDEXA, 14 women were scanned in triplicate on both devices within 1 mo. All measurements included in the present analysis were highly correlated among both devices ($r > 0.87$). For cross-calibration between the Hologic QDR4500W and Lunar iDXA, 32 women were scanned in duplicate on both devices on the same day. High correlations among both devices were observed for all measurements included in the present analysis ($r > 0.93$). To remove system bias, values obtained from the Lunar Prodigy and Hologic QDR- 4500W were calibrated to the Lunar iDEXA.

*Modeling of organ tissue mass.* Following whole body DEXA analysis, the organ tissue mass of the brain, skeletal muscle, bone, adipose tissue, and residual tissue were determined as follows (25):

$$\text{Brain mass} = 0.005 \times \text{skull area (in cm}^2\text{)} + 0.2 \times \text{sex*} + 0.24,$$

$$\text{Skeletal muscle mass} = 1.13 \times \text{lean tissue in extremities (kg)} - 0.02 \times \text{age (yr)} + 0.61 \times \text{sex} + 0.97$$

$$\text{Bone mass} = 1.85 \times \text{bone mineral content (kg)}$$

$$\text{Adipose tissue mass} = 1.18\_ \text{ fat mass (kg)}$$

where sex (*) = 0 for female and sex = 1 for male.

Residual mass was defined as the difference between total body mass and the sum of brain, skeletal muscle, bone, and adipose tissue mass (25).

Skull area was defined as head area ($cm^2$) obtained from whole body ancillary results and referred to the area superior to the bottom of the mandible. Lean mass (kg) in the extremities was obtained from body composition results and referred to the area lateral to the glenohumeral joint (arms) and distal to the femoral neck (legs). Bone mineral content and fat mass were obtained from whole body analyses.

*Prediction of resting metabolic rate from organ tissue mass.* Using previously published tissue coefficients of 240 $kcal \cdot kg^{-1} \cdot day^{-1}$ for brain, 13 $kcal \cdot kg^{-1} \cdot day^{-1}$ for skeletal muscle, 4.5 $kcal \cdot kg^{-1} \cdot day^{-1}$ for adipose tissue, 2.3 $kcal \cdot kg^{-1} \cdot day^{-1}$ for bone, and 43 $kcal \cdot kg^{-1} \cdot day^{-1}$ for residual mass (16, 25), tissue-specific metabolic rates were calculated as the product of the organ tissue mass and the respective tissue coefficients. DEXA-predicted RMR was calculated as the sum of the tissue-specific metabolic rates of brain, skeletal muscle, adipose tissue, bone, and residual mass.

*Metabolic hormones.* Following an overnight fast of ≥12 h and abstention from exercise and caffeine for 24 h, blood samples were collected between 0700 and 1000 on one to two occasions during or immediately following the menstrual status monitoring period. As described previously (8), samples were pooled prior to analysis, and serum concentrations of total triiodothyronine ($TT_3$) were analyzed using a chemiluminescence immunoassay analyzer (Immulite; Diagnostic Products, Los Angeles, CA) with a sensitivity of 35 ng/dl and intra- and interassay coefficients of variation of 13.2 and 15.6%, respectively. Serum leptin was measured using an ELISA (Millipore, St. Charles, MI) with a sensitivity of 0.5 ng/ml and inter- and intra-assay coefficients of variations of 6.2 and 4.6%, respectively. All samples were measured in duplicate.

*Statistical analyses.* Statistical analyses were performed with R (version 2.14.1). If not stated otherwise, data were reported as means ± SE. Normality was assessed using the Shapiro-Wilk test. For normally distributed variables, group differences between the AMEN group and the OV group and difference among measured and predicted RMR within groups were assessed using one-way analysis of variance. For not normally distributed data, the Kruskal-Wallis test was used to identify differences. Spearman's correlation coefficient ($\rho$) was assessed to determine associations between RMR and serum $TT_3$ and leptin concentrations. The level of significance ($\alpha$) was set at $P < 0.05$.

## Results

*Participants.* Among the 287 women who were initially enrolled in the two original studies, 208 were excluded because of incomplete data or because they did not meet the criteria for inclusion in this study or our criteria for menstrual status classification (Fig. 1). Among the remaining 79 women, 42 qualified as AMEN and 37 qualified as OV. AMEN women were slightly younger ($P = 0.010$) and taller ($P = 0.005$) and exhibited a lower body mass index ($P = 0.028$) and body fat percentage ($P = 0.024$) when compared with OV women (Table 1). There were no significant differences between the AMEN group and the OV group with respect to body mass, fat mass, lean mass, aerobic fitness, and self-reported weekly exercise. The primary type of exercise involved aerobic exercise (running, cycling, cardio training, swimming, triathlon, or aerobics) for the majority of the women (72%). The remaining women conducted resistance exercise (4%) or exercise involving both aerobic and resistance components (24%), such as soccer, dance, tennis, pentathlon, field hockey, lacrosse, or both aerobic and strength training. Primary type of exercise was not different between the AMEN group and the OV group ($\chi^2 = 1.22$, $P = 0.58$).

*Menstrual characteristics.* Menarche occurred at a slightly older age in the AMEN group compared with the OV group (13.1 ± 0.2 vs. 12.2 ± 0.2, $P = 0.002$). At the time of the study, AMEN women had been amenorrheic for 292 ± 39 days (range: 90–1,642 days). In the OV group, the average menstrual cycle length was 29.6 ± 0.4 days, with follicular and luteal phases of 18.9 ± 0.5 and 10.7 ± 0.3 days, respectively. Five women in the OV group (13%) demonstrated luteal phase defects, which were attributed to short luteal phases ($n = 3$) or a combination of short and inadequate luteal phases ($n = 2$). The AMEN group demonstrated chronically suppressed concentrations of urinary E1G and PdG throughout the 28-day monitoring period, whereas the OV group exhibited characteristic late-follicular and luteal rises in E1G and PdG, respectively (Fig. 2). When compared with the OV group, the AMEN group demonstrated lower mean concentrations of E1G (25.0 ± 1.7 vs. 46.2 ± 3.4 ng/ml, $P < 0.001$) and PdG (1.0 ± 0.1 vs. 2.5 ± 0.2 μg/ml, $P < 0.001$) as well as lower areas under the curve for E1G (678 ± 48 vs. 1,316 ± 95 $ng \cdot day^{-1} \cdot ml^{-1}$, $P < 0.001$) and PdG (26.3 ± 2.3 vs. 72.6 ± 4.7 $\mu g \cdot day^{-1} \cdot ml^{-1}$).

*Comparison of measured and predicted resting metabolic rate.* Measured RMR was not significantly lower in the AMEN group when compared with the OV group when expressed in absolute numbers ($P = 0.08$) but was lower when expressed relative to lean mass ($P < 0.001$; Table 2). In contrast, DEXA-predicted RMR was significantly higher in the AMEN group when compared with the OV group ($P = 0.004$). Consequently, in the AMEN group, measured RMR was, on average, 112 ± 26 kcal/day lower than DEXA-predicted RMR ($P < 0.001$; Table 2), whereas measured and DEXA-predicted RMR were similar in the OV group ($P = 0.16$). The ratio of measured to DEXA-predicted RMR (Fig. 3) was significantly lower in the AMEN group (92 ± 2%) when compared with the OV group (103 ± 2%, $P < 0.001$).

*Size of metabolically active tissue compartments.* On average, the AMEN group exhibited 1.6 kg less adipose tissue ($P = 0.008$) and 1.7 kg more residual mass ($P < 0.001$) when compared with OV group (Table 3). The differences between the AMEN group and the OV group with regard to adipose tissue ($P = 0.005$) and residual mass ($P < 0.001$) remained significant even after the size of metabolic tissue compartments for body mass was normalized.

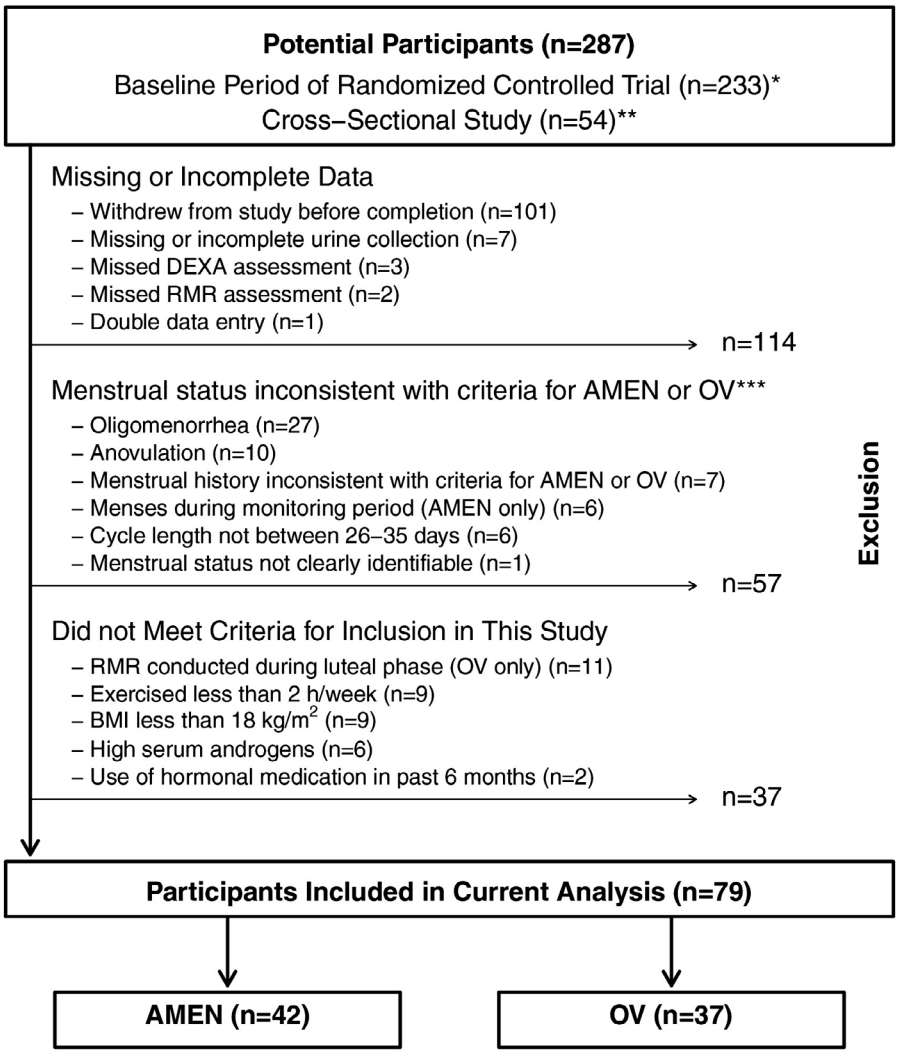


**Fig. 1.** Inclusion and exclusion of participants. *See Ref. 33. **See Ref. 15. ***Exercising women with amenorrhea (AMEN) were defined by self-reported absence of menses for ≥90 days and chronically suppressed urinary estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG) throughout the monitoring period (14); exercising women with ovulatory cycles (OV) were defined by ≥9 self-reported menses in the past year, a monitored menstrual cycle length between 26 and 35 days, an E1G peak concentration >35 ng/ll, an LH surge concentration >25 mIU/ml occurring on or after the day of the E1G peak, and a PdG concentration >2.5 μg/ml during the luteal phase (14, 15). DEXA, dual-energy X-ray absorptiometry; RMR, resting metabolic rate; BMI, body mass index.

There were no significant differences in the absolute and relative sizes of brain, skeletal muscle, or bone mass between the AMEN group and the OV group.

**Table 1.** Anthropometric and exercise characteristics of the study participants

| Characteristic | AMEN (*n* = 42) | OV (*n* = 37) |
|---|---|---|
| Age, yr | 22.3 ± 0.5 | 24.7 ± 0.7* |
| Height, cm | 168 ± 1 | 164 ± 1** |
| Body mass, kg | 58.4 ± 1.0 | 58.2 ± 0.8 |
| Body mass index, kg/m$^2$ | 20.8 ± 0.3 | 21.6 ± 0.2* |
| Fat mass, kg | 12.7 ± 0.7 | 13.9 ± 0.5 |
| Body fat, % | 21.5 ± 0.9 | 24.1 ± 0.8* |
| Lean body mass, kg | 43.1 ± 0.8 | 41.1 ± 0.6 |
| Lean mass index, kg/m$^2$ | 15.3 ± 0.2 | 15.2 ± 0.2 |
| V ˙ O2peak, ml·kg$^{-1}$·min$^{-1}$† | 49.3 ± 1.6 | 47.1 ± 1.3 |
| Self-reported exercise, min/wk | 450 ± 44 | 396 ± 34 |

Values are means ± SE. AMEN, women with exercise-associated amenorrhea; OV, exercising women with ovulatory menstrual cycles; $\dot{V}_{O2peak}$, peak oxygen uptake. *$P$ < 0.05 vs. AMEN; **$P$ < 0.01 vs. AMEN; †sample size for $\dot{V}_{O2peak}$: $n$ = 32 AMEN and = 32 OV.

*Association of metabolic and reproductive hormones with resting metabolic rate.* When compared with the OV group, the AMEN group demonstrated lower serum concentrations of $TT_3$ (80.6 ± 3.3 vs. 90.6 ± 2.2 ng/dl, $P$ = 0.012) and leptin (5.1 ± 0.9 vs. 7.9 ± 0.8 ng/ml, $P$ < 0.001). The difference in leptin among the AMEN group and the OV group remained significant even when serum leptin was adjusted for the size of adipose tissue (0.28 ± 0.04 ng·ml$^{-1}$·kg$^{-1}$ vs. 0.42 ± 0.04, $P$ _ 0.001). The ratio of measured to DEXA-predicted RMR was significantly correlated to serum $TT_3$ ($\rho$ = 0.38, $P$ < 0.001; Fig. 4) and leptin ($\rho$ = 0.32, $P$ = 0.004; Fig. 4). There was no significant correlation between the ratio of measured to DEXA-predicted RMR and mean concentrations as well as areas under the curve for E1G and PdG (all $P$ > 0.14).

## Discussion

The primary finding of this cross-sectional study was that, after accounting for differences in the size of the main metabolic tissue compartments, measured RMR in exercising women with amenorrhea was on average 8% lower than predicted, whereas there was no difference between measured and predicted RMR in exercising women with ovulatory menstrual cycles. Consistent with the

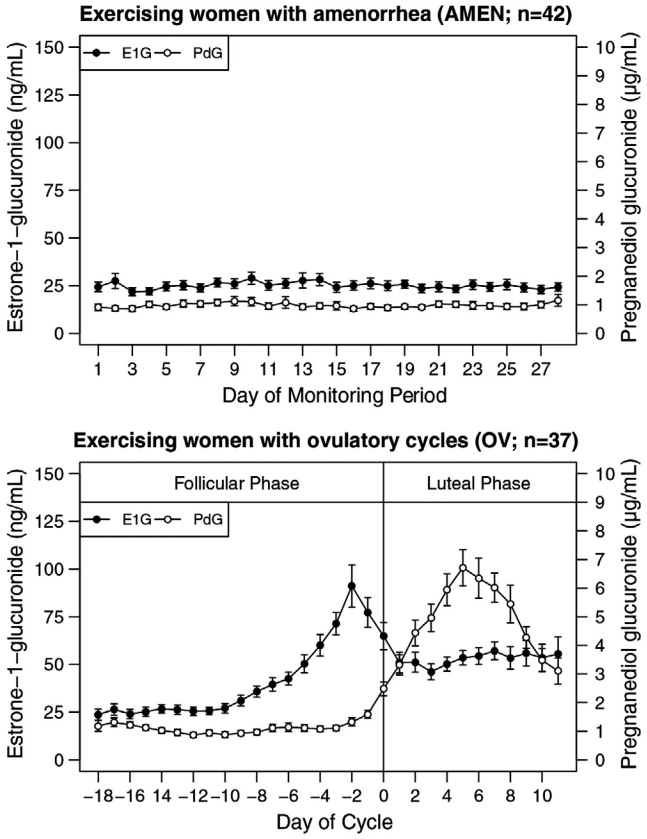


**Fig. 2.** Composite graphs of urinary estrone-1-glucuronide (E1G; ●) and pregnanediol glucuronide (PdG; ○) across the 28-day monitoring period in exercising women with amenorrhea (AMEN; *top*) and across 1 menstrual cycle in exercising women with ovulatory cycles (OV; *bottom*).

lower than predicted RMR, exercising women with amenorrhea also demonstrated reduced serum concentrations of $TT_3$ and leptin compared with exercising women with ovulatory cycles. Furthermore, the size of tissue compartments with high metabolic activity (brain, skeletal muscle, and residual mass) was not reduced in exercising women with amenorrhea, suggesting that low RMR in this population is not explained by an altered tissue composition. Rather, the suppressed RMR in exercising women with amenorrhea may be attributed to alterations in the metabolic activity of tissues. Taken together, these findings are important because they suggest that women with exercise-associated amenorrhea suffer from metabolic suppression as an adaptive response to conserve energy.

**Table 2.** Measured and predicted RMR among AMEN and OV women

| RMR | AMEN ($n$ = 42) | OV ($n$ = 37) |
|---|---|---|
| Measured, kcal/day | 1,215. ± 31 | 1,284. ± 24 |
| Measured, kcal·kg $LBM^{-1}$·$day^{-1}$ | 28.3 ± 0.6 | 31.3 ± 0.6*** |
| DEXA-predicted, kcal/day | 1,327. ± 18### | 1,252. ± 17** |

Values are means ± SE. RMR, resting metabolic rate; AMEN, women with exercise-associated amenorrhea; OV, exercising women with ovulatory menstrual cycles; LBM, lean body mass; DEXA, dual-energy X-ray absorptiometry. ###$P$ < 0.001 vs. measured; **$P$ < 0.01 vs. AMEN; ***$P$ < 0.001 vs. AMEN.

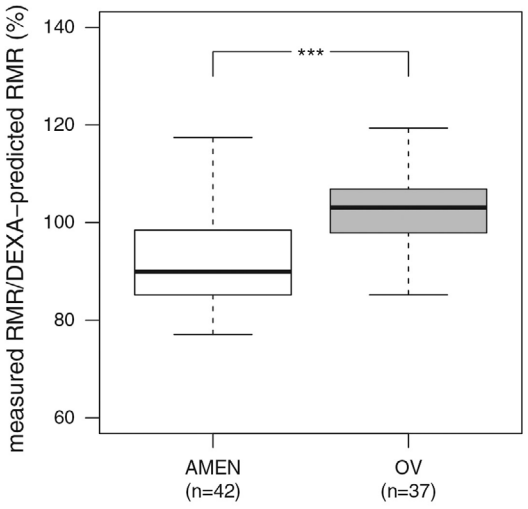


**Fig. 3.** Comparison of the ratio of measured to DEXA-predicted RMR among women with exercise-associated amenorrhea (AMEN) and exercising women with ovulatory menstrual cycles (OV). Bold horizontal lines denote the median. *Bottom* and *top* of the boxes indicate the 1st and 3rd quartile, respectively, and dashed lines denote the interquartile range. ***$P$ < 0.001.

Our findings are in agreement with previous longitudinal experiments documenting that RMR decreases once an energy deficit is induced (23). Because this RMR reduction normally exceeds the reduction in RMR expected from lean tissue losses, it is understood as an adaptive response to conserve energy (3, 4, 36, 41). Although a reduction in RMR is easily detectable in longitudinal settings, it is far more challenging to characterize RMR reductions cross-sectionally. Utilizing a novel approach that accounts for differences in the size of metabolic tissue compartments as determined by DEXA, we were able to demonstrate that RMR is suppressed in chronically energy-deficient exercising women with amenorrhea, whereas using the same approach we found that measured and predicted RMR were in good agreement in our control group of ovulatory exercising women. To our knowledge, a similar approach has been utilized only in anorexia nervosa patients, in whom measured RMR was also lower than DEXA-predicted RMR (30). Because anorexia nervosa presents a model of severe energy deficiency (39), it is not surprising that the 21% discrepancy between measured and DEXA-predicted RMR in these patients was more pronounced compared with the 8% reduction in our group of exercising women with amenorrhea, which did not include women with clinical eating disorders. Furthermore, since exercise has been shown to attenuate the reduction in RMR during calorie-restricted weight loss (44), their habitual exercise of ~7.5 h/wk may have protected our women from greater reductions in RMR.

The present study further expands upon previous reports of low RMR in women with EAMD, in whom RMR is 14–20% lower than predicted by the Harris-Benedict equation (10, 15, 21). However, the Harris-Benedict equation, which predicts RMR from height, weight, and age (24), does not account for differences in body tissue composition at all. To normalize RMR, some authors have expressed RMR relative to the size of lean mass and reported a 5–15% reduction in lean mass-adjusted RMR in women with EAMD (10, 15, 21) and a 12–30% reduction in lean mass-adjusted RMR in anorexia nervosa patients (32, 38, 40). However, this approach is also problematic because the contribution of lean mass

**Table 3.** Absolute and relative size of the main metabolically active body compartments among AMEN and OV women

| Compartment | Absolute Size, kg | | Relative Size, %total mass | |
|---|---|---|---|---|
| | AMEN (*n* = 42) | OV (*n* = 37) | AMEN (*n* = 42) | OV (*n* = 37) |
| Brain mass | 1.4 ± 0.0 | 1.4 ± 0.0 | 2.4 ± 0.0 | 2.4 ± 0.0 |
| Skeletal muscle mass | 21.8 ± 0.5 | 21.2 ± 0.4 | 37.6 ± 0.5 | 36.9 ± 0.5 |
| Adipose mass | 16.2 ± 0.7 | 17.8 ± 0.5** | 27.5 ± 0.9 | 30.9 ± 0.7** |
| Bone mass | 4.4 ± 0.1 | 4.5 ± 0.1 | 7.6 ± 0.1 | 7.8 ± 0.1 |
| Residual mass | 14.5 ± 0.3 | 12.8 ± 0.3*** | 25.1 ± 0.5 | 22.3 ± 0.4*** |

Values are means ± SE. AMEN, women with exercise-associated amenorrhea; OV, exercising women with ovulatory menstrual cycles; ** $P < 0.01$ vs. AMEN; *** $P < 0.001$ vs. AMEN.

to RMR is not constant (48). Although lean mass accounts for almost two-thirds of the variance in RMR in adults (27), it is composed of various tissues with highly variable metabolic rates (25). Vital compartments such as the brain or inner organs require considerably more energy compared with nonvital compartments of lean mass such as skeletal muscle (25). As a consequence, RMR, when expressed in kcal/kg lean mass, is naturally higher in individuals with low lean mass and a high proportion of vital tissues compared with individuals with high lean mass and a high proportion of nonvital tissues (48). As such, approaches that fail to account for the contribution of these different metabolic tissue compartments could lack the sensitivity to detect RMR suppression and characterize the full magnitude of metabolic adaptations.

In fact, we observed significant differences in the size of these compartments among AMEN and OV after we divided lean mass into its three major tissue compartments (skeletal muscle, brain, and residual mass). When expressed relative to body size, the AMEN group exhibited on average 13% more residual mass, which is reflective of inner organ tissue such as heart, liver, and kidney as well as other soft tissues (25). These vital tissue compartments are typically preserved during chronic energy deficiency (22) so that the greater proportion of residual mass in AMEN is likely a result of the relative reduction in the size of other tissue compartments. Because the degradation of tissue to supply additional energy is limited mainly to easily expendable compartments (22), it is not surprising that adipose tissue mass was reduced in AMEN. However, the AMEN group did not demonstrate reduced skeletal muscle mass, another expendable tissue that can provide energy and, in particular, glucose to meet energy requirements of the brain (5, 17). The apparent preservation of skeletal muscle mass in our group of AMEN is likely attributable to their exercise, which has the capacity to attenuate muscle loss during energy deficiency (44).

The association between metabolic adaptations indicative of energy conservation and the induction and reversal of exercise-induced amenorrhea secondary to changes in energy status has been demonstrated in both animal and human models. When female cynomolgus monkeys were exposed to an energy deficit through increasing their exercise energy expenditure, they exhibited a 20% reduction in circulating $TT_3$, which occurred concomitant with the onset of amenorrhea (50). Likewise, when dietary energy was increased to compensate for the increased energy expenditure of exercise, circulating TT3 returned to baseline levels, and the increase in $TT_3$ coincided with the onset of menses (50). Leptin is another key regulator of energy balance that is suppressed during energy deficiency induced by caloric restriction and/or increased exercise (28, 31). The importance of leptin for reproductive function in women is highlighted by findings that the suppression of leptin secondary to energy deficiency is concomitant with a suppression of LH pulsatility (31) and that administration of human recombinant leptin can revert the suppression of the hypothalamic- pituitary-gonadal axis during energy deficiency (6) and results in the recovery of menses in previously amenorrheic women (7). Given the correlation between $TT_3$, leptin, and the ratio of measured to DEXA-predicted RMR reported in the present study, it is likely that changes in these metabolic hormones during the transition between normal and suppressed reproductive function are paralleled by characteristic changes in RMR.

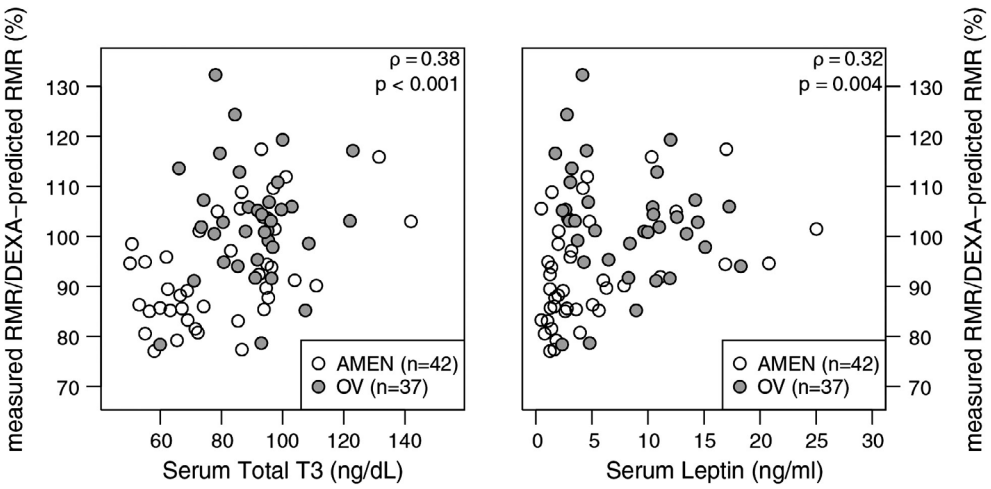


**Fig. 4.** Correlations between the ratio of measured to DEXA-predicted RMR and serum concentrations of total triiodothyronine (*left*) and leptin (*right*) among women with exercise-associated amenorrhea (AMEN) and exercising women with ovulatory menstrual cycles (OV).

Our results further inform our understanding of the energy cost of human reproduction and, notably, that of normal menstrual function. The 8% reduction in RMR in women with completely absent menstrual cycles confirms that maintenance of normal menstrual function is an energy-demanding process (26). Because of this energy cost, it has been hypothesized that the loss of menstrual function can act to conserve energy (26). However, to this day, the true energy cost of menstrual function remains largely unknown (26). Previously, estimations of energy requirements were based on metabolic rate fluctuations across the menstrual cycle, which can be as high as 7–9% (2, 37, 47). These transient changes in RMR across the menstrual cycle have been attributed to an increase in progesterone during the luteal phase (47). To date, the reduction in RMR caused by the complete suppression of menstrual function has been assessed in only one study. Following ovarian suppression via administration of a gonadotropin-releasing hormone antagonist, RMR was reduced by 3–5% compared with RMR measured in the same women when they were menstruating regularly (9). However, the pharmacological intervention lasted only 6 days and did not address long-term RMR changes following the loss of menstrual function. The fact that the 8% RMR suppression in our sample exceeded the short-term reduction in RMR following acute ovarian suppression, together with the lack of a significant association between RMR suppression and urinary measurements of progesterone and estrogen exposure, suggests that the suppression of RMR in exercising women with amenorrhea is not caused exclusively by reduced circulating ovarian steroids. Instead, these findings further support our hypothesis that RMR suppression in this group is the result of metabolic adaptations in response to chronic energy deficiency.

Our findings and approach need to be confirmed in future longitudinal trials in humans. A recent case study from our laboratory found that RMR increased in two recreational athletes with EAMD who resumed menses while participating in a year-long intervention of increased caloric intake (35). In both women, the menstrual recovery coincided with an increase in RMR (35). Since both women also demonstrated an increase in fat mass, it is likely that these changes in RMR would have been even more pronounced after accounting for changes in the size of metabolically active tissue compartments secondary to the intervention. Another limitation of the present study was that the use of DEXA restricted us to assessing residual mass as a surrogate of organ tissue mass as opposed to measuring the size of individual organs. RMR prediction could be improved further by accounting for differences in the size of highly active metabolic organs such as the liver, heart, or kidneys (16). In fact, others have utilized whole body magnetic resonance imaging (MRI) to assess organ size and predict RMR (18, 46). However, in contrast to MRI, DEXA is widely used in clinical and research settings and is associated with lower costs (25).

In summary, our results demonstrate that even after the prediction of whole body RMR is improved by accounting for differences in the size of tissue compartments of varying metabolic rates, RMR remained suppressed in exercising women with amenorrhea. These findings suggest that RMR suppression in women with exercise-associated amenorrhea is the result of a reduced metabolic activity at the tissue level, thus providing further evidence of energy conservation in this population.

**Acknowledgments —** This study was supported by the U.S. Department of Defense (PR054531). K. Koehler was supported through a Research Fellowship awarded by the German Academic Exchange Service.

**Author Contributions —** K.K., N.I.W., R.J.M., and M.J.D.S. conception and design of research; K.K., R.J.M., E.A.S., and H.C.A. performed experiments; K.K., R.J.M., E.A.S., and H.C.A. analyzed data; K.K., N.I.W., R.J.M., and M.J.D.S. interpreted results of experiments; K.K. and R.J.M. prepared figures; K.K. and R.J.M. drafted manuscript; K.K., N.I.W., R.J.M., and M.J.D.S. edited and revised manuscript; K.K., N.I.W., R.J.M., E.A.S., H.C.A., and M.J.D.S. approved final version of manuscript.

## References

1. Practice Committee of American Society for Reproductive Medicine. Current evaluation of amenorrhea. *Fertil Steril* 90 (5 *Suppl*): S219–S225, 2008.
2. Bisdee JT, James WP, Shaw MA. Changes in energy expenditure during the menstrual cycle. *Br J Nutr* 61: 187–199, 1989.
3. Bouchard C, Tremblay A, Despres JP, Theriault G, Nadeau A, Lupien PJ, Moorjani S, Prudhomme D, Fournier G. The response to exercise with constant energy intake in identical twins. *Obes Res* 2: 400–410, 1994.
4. Byrne NM, Wood RE, Schutz Y, Hills AP. Does metabolic compensation explain the majority of less-than-expected weight loss in obese adults during a short-term severe diet and exercise intervention? *Int J Obes (Lond)* 36: 1472–1478, 2012.
5. Cahill GF Jr. Starvation in man. *Clin Endocrinol Metab* 5: 397–415, 1976.
6. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111: 1409–1421, 2003.
7. Chou SH, Chamberland JP, Liu X, Matarese G, Gao C, Stefanakis R, Brinkoetter MT, Gong H, Arampatzi K, Mantzoros CS. Leptin is an effective treatment for hypothalamic amenorrhea. *Proc Natl Acad Sci USA* 108: 6585–6590, 2011.
8. Corr M, De Souza MJ, Toombs RJ, Williams NI. Circulating leptin concentrations do not distinguish menstrual status in exercising women. *Hum Reprod* 26: 685–694, 2011.
9. Day DS, Gozansky WS, Van Pelt RE, Schwartz RS, Kohrt WM. Sex hormone suppression reduces resting energy expenditure and {beta}-adrenergic support of resting energy expenditure. *J Clin Endocrinol Metab* 90: 3312–3317, 2005.
10. De Souza MJ, Lee DK, VanHeest JL, Scheid JL, West SL, Williams NI. Severity of energy-related menstrual disturbances increases in proportion to indices of energy conservation in exercising women. *Fertil Steril* 88: 971–975, 2007.
11. [deleted]
12. De Souza MJ, Leidy HJ, O'Donnell E, Lasley B, Williams NI. Fasting ghrelin levels in physically active women: relationship with menstrual disturbances and metabolic hormones. *J Clin Endocrinol Metab* 89: 3536–3542, 2004.
13. De Souza MJ, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, Gibbs JC, Olmsted M, Goolsby M, Matheson G. 2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. *Br J Sports Med* 48: 289, 2014.
14. De Souza MJ, Toombs RJ, Scheid JL, O'Donnell E, West SL, Williams NI. High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. *Hum Reprod* 25: 491–503, 2010.
15. De Souza MJ, West SL, Jamal SA, Hawker GA, Gundberg CM, Williams NI. The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone* 43: 140–148, 2008.

16. Elia M. Organ and tissue contribution to metabolic rate. In: *Energy Metabolism: Tissue Determinants and Cellular Corollaries*, edited by Kinney JM Tucker HN. New York: Raven, 1992, p. 61–79.
17. Finn PF, Dice JF. Proteolytic and lipolytic responses to starvation. *Nutrition* 22: 830–844, 2006.
18. Gallagher D, Belmonte D, Deurenberg P, Wang Z, Krasnow N, Pi-Sunyer FX, Heymsfield SB. Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. *Am J Physiol Endocrinol Metab* 275: E249–E258, 1998.
19. Garner DM, Olmstead MP. *Eating Disorder Inventory Manual*. Odessa, FL: Psychological Assessment Resources, 1991.
20. Gibbs JC, Williams NI, De Souza MJ. Prevalence of individual and combined components of the female athlete triad. *Med Sci Sports Exerc* 45: 985–996, 2013.
21. Gibbs JC, Williams NI, Scheid JL, Toombs RJ, Souza MJ. The association of a high drive for thinness with energy deficiency and severe menstrual disturbances: confirmation in a large population of exercising women. *Int J Sport Nutr Exerc Metab* 21: 280–290, 2011.
22. Hall KD. Computational model of in vivo human energy metabolism during semistarvation and refeeding. *Am J Physiol Endocrinol Metab* 291: E23–E37, 2006.
23. Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL, Swinburn BA. Quantification of the effect of energy imbalance on bodyweight. *Lancet* 378: 826–837, 2011.
24. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci USA* 4: 370–373, 1918.
25. Hayes M, Chustek M, Wang Z, Gallagher D, Heshka S, Spungen A, Bauman W, Heymsfield SB. DXA: potential for creating a metabolic map of organ-tissue resting energy expenditure components. *Obes Res* 10: 969–977, 2002.
26. Jasienska G. Energy metabolism and the evolution of reproductive suppression in the human female. *Acta Biotheor* 51: 1–18, 2003.
27. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *Am J Clin Nutr* 82: 941–948, 2005.
28. Koehler K, Hoerner NR, Gibbs JC, Zinner C, Braun H, De Souza MJ, Schaenzer W. Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones. *J Sports Sci*: 1–9, 2016 [Epub ahead of print].
29. Konrad KK, Carels RA, Garner DM. Metabolic and psychological changes during refeeding in anorexia nervosa. *Eat Weight Disord* 12: 20–26, 2007.
30. Kosmiski L, Schmiege SJ, Mascolo M, Gaudiani J, Mehler PS. Chronic starvation secondary to anorexia nervosa is associated with an adaptive suppression of resting energy expenditure. *J Clin Endocrinol Metab* 99: 908–914, 2014.
31. Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab* 88: 297–311, 2003.
32. Luke A, Schoeller DA. Basal metabolic rate, fat-free mass, and body cell mass during energy restriction. *Metabolism* 41: 450–456, 1992.
33. Mallinson RJ, Williams NI, Gibbs JC, Koehler K, Allaway HC, Southmayd E, De Souza MJ. Current and past menstrual status is an important determinant of femoral neck geometry in exercising women. *Bone* 88: 101–112, 2016.
34. Mallinson RJ, Williams NI, Hill BR, De Souza MJ. Body composition and reproductive function exert unique influences on indices of bone health in exercising women. *Bone* 56: 91–100, 2013.
35. Mallinson RJ, Williams NI, Olmsted MP, Scheid JL, Riddle ES, De Souza MJ. A case report of recovery of menstrual function following a nutritional intervention in two exercising women with amenorrhea of varying duration. *J Int Soc Sports Nutr* 10: 34, 2013.
36. Martin CK, Heilbronn LK, de Jonge L, DeLany JP, Volaufova J, Anton SD, Redman LM, Smith SR, Ravussin E. Effect of calorie restriction on resting metabolic rate and spontaneous physical activity. *Obesity (Silver Spring)* 15: 2964–2973, 2007.
37. Meijer GA, Westerterp KR, Saris WH, ten Hoor F. Sleeping metabolic rate in relation to body composition and the menstrual cycle. *Am J Clin Nutr* 55: 637–640, 1992.
38. Melchior JC, Rigaud D, Rozen R, Malon D, Apfelbaum M. Energy expenditure economy induced by decrease in lean body mass in anorexia nervosa. *Eur J Clin Nutr* 43: 793–799, 1989.
39. Misra M, Klibanski A. The neuroendocrine basis of anorexia nervosa and its impact on bone metabolism. *Neuroendocrinology* 93: 65–73, 2011.
40. Polito A, Fabbri A, Ferro-Luzzi A, Cuzzolaro M, Censi L, Ciarapica D, Fabbrini E, Giannini D. Basal metabolic rate in anorexia nervosa: relation to body composition and leptin concentrations. *Am J Clin Nutr* 71: 1495–1502, 2000.
41. Redman LM, Heilbronn LK, Martin CK, de Jonge L, Williamson DA, Delany JP, Ravussin E. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. *PLoS One* 4: e4377, 2009.
42. Scheid JL, Williams NI, West SL, VanHeest JL, De Souza MJ. Elevated PYY is associated with energy deficiency and indices of subclinical disordered eating in exercising women with hypothalamic amenorrhea. *Appetite* 52: 184–192, 2009.
43. [deleted]
44. Thomas DM, Bouchard C, Church T, Slentz C, Kraus WE, Redman LM, Martin CK, Silva AM, Vossen M, Westerterp K, Heymsfield SB. Why do individuals not lose more weight from an exercise intervention at a defined dose? An energy balance analysis. *Obes Rev* 13: 835–847, 2012.
45. Wade GN, Schneider JE, Li HY. Control of fertility by metabolic cues. *Am J Physiol Endocrinol Metab* 270: E1–E19, 1996.
46. Wang Z, Heshka S, Gallagher D, Boozer CN, Kotler DP, Heymsfield SB. Resting energy expenditure-fat-free mass relationship: new insights provided by body composition modeling. *Am J Physiol Endocrinol Metab* 279: E539–E545, 2000.
47. Webb P. 24-hour energy expenditure and the menstrual cycle. *Am J Clin Nutr* 44: 614–619, 1986.
48. Weinsier RL, Schutz Y, Bracco D. Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *Am J Clin Nutr* 55: 790–794, 1992.
49. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 109: 1–9, 1949.
50. Williams NI, Caston-Balderrama AL, Helmreich DL, Parfitt DB, Nosbisch C, Cameron JL. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: abrupt transition to exercise-induced amenorrhea. *Endocrinology* 142: 2381–2389, 2001.