Low energy availability, not stress of exercise, alters LH pulsatility in exercising women

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Loucks, A. B., M. Verdun, and E. M. Heath. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J. Appl. Physiol. 84(1): 37–46, 1998.—We tested two hypotheses about the disruption of luteinizing hormone (LH) pulsatility in exercising women by assaying LH in blood samples drawn at 10-min intervals over 24 h from nine young, habitually sedentary, regularly menstruating women on days 8, 9, or 10 of two menstrual cycles after 4 days of intense exercise \( [E = 30 \text{ kcal-kg lean body mass (LBM)}^{-1}\cdot\text{day}^{-1} \text{ at } 70\% \text{ of aerobic capacity}] \). To test the hypothesis that LH pulsatility is disrupted by low energy availability, we controlled the subjects' dietary energy intakes \( (I) \) to set their energy availabilities \( (A = I - E) \) at 45 and 10 kcal-kg LBM \( ^{-1}\cdot\text{day}^{-1} \) during the two trials. To test the hypothesis that LH pulsatility is disrupted by the stress of exercise, we compared the resulting LH pulsativities to those previously reported in women with similar controlled energy availability who had not exercised. In the exercising women, low energy availability reduced LH pulse frequency by 10% \( (P < 0.01) \) during the waking hours and increased LH pulse amplitude by 36% \( (P = 0.05) \) during waking and sleeping hours, but this reduction in LH pulse frequency was blunted by 60% \( (P = 0.03) \) compared with that in the previously studied nonexercising women whose low energy availability was caused by dietary restriction. The stress of exercise neither reduced LH pulse frequency nor increased LH pulse amplitude \( (P < 0.4) \). During exercise, the proportion of energy derived from carbohydrate oxidation was reduced from 73% while \( A = 45 \text{ kcal-kg LBM}^{-1}\cdot\text{day}^{-1} \) to 49% while \( A = 10 \text{ kcal-kg LBM}^{-1}\cdot\text{day}^{-1} \) \( (P < 0.003) \). These results contradict the hypothesis that LH pulsatility is disrupted by exercise stress and suggest that LH pulsatility in women depends on energy availability.

athletic amenorrhea; nutrition; reproduction; metabolic hormones; luteinizing hormone

athletic women display a disproportionately high prevalence of amenorrhea (see Ref. 17 for review). Endocrine and neuroendocrine experiments have found that the proximal cause of menstrual and ovarian dysfunction in these women is disruption of the pulsatile secretion of luteinizing hormone (LH) by the pituitary and that this is caused by disruption of the pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus (19). Animal experiments and clinical observations indicate that GnRH and LH pulsatility are not disrupted by low body weight or by an excessively lean body composition in amenorrheic athletes (see Ref. 2 for review). Rather, GnRH and LH pulsatility appear to be disrupted either by the stress of exercise or by low energy availability (Ref. 11).

The so-called "exercise stress hypothesis" holds that exercise training is a chronic stressor that, like other chronic stressors, activates the hypothalamic-pituitary-adrenal (HPA) axis and that one or more of the central and peripheral mediators of the HPA axis disrupts the GnRH pulse generator. This hypothesis is supported by in vitro and in vivo demonstrations of neuroendocrine agents disrupting GnRH and LH pulsatility, by mild hypercortisolism and altered ACTH regulation in amenorrheic athletes (13, 19), and by experiments in which reproductive function in animals was disrupted by various stressors (22) including exercise training (e.g., Refs. 4 and 24). If this hypothesis is true, the appropriate intervention to prevent or reverse menstrual disorders in athletes is to moderate the exercise regimen.

The so-called "energy availability hypothesis" holds that the GnRH pulse generator is disrupted by an as-yet-unidentified signal that dietary energy intake is inadequate for the energy costs of both reproduction and locomotion. That hypothesis is supported by reports that athletic women consume less energy than would be expected for their activity level; by endocrine signs of chronic energy deficiency in amenorrheic athletes; by similarities between athletic amenorrhea, weight-loss amenorrhea, and anorexia nervosa; and by an extensive literature on the bioenergetics of reproduction. This literature has documented a dependence of reproductive function on energy availability in mammalian species ranging from rodents to humans (see Refs. 30 and 31 for reviews). If this hypothesis is true, then athletes may be able to prevent or reverse menstrual disorders by increasing dietary energy intake without moderating their exercise regimen.

Other investigators have reported that the combination of short-term strenuous exercise and caloric restriction suppresses LH pulse frequency in women (32). The purpose of this experiment was to differentiate the independent effects of energy availability and exercise stress on LH pulsatility. These factors had been confounded in previous observational and experimental investigations of the influence of exercise on reproductive function in both animal models and humans. This goal required objective and operational definitions that would enable us to control these factors independently. The bioenergetics literature (30, 31) suggested such a definition for energy availability (dietary energy intake – exercise energy expenditure), but we were unable to discern a similarly objective and operational definition for exercise stress anywhere in the extensive literature that has characterized the physiological responses to exercise. Investigators of stress define stress broadly as "any stimulus that disturbs the homeostasis of the organism" (22). From their viewpoint, our aim...
was not to test mutually exclusive stress and energy availability hypotheses but rather to test whether low energy availability or exercise is the particular stressor that disrupts LH pulsatility in women who exercise. Therefore, for this experiment, we defined the stress of exercise objectively, operationally, and independently of energy availability as everything associated with exercise except its energy cost.

In the previously reported (16) first phase of the present experiment (Fig. 1), we reduced LH pulse frequency and increased LH pulse amplitude in habitually sedentary women by restricting their dietary energy intake from 45 to 10 kcal·kg lean body mass (LBM)−1·day−1 by means of dietary energy restriction (16). Those women had achieved menarche 1 yr later (13.2 ± 0.2 yr; P < 0.05) from the previously reported nonexercising women under similar energy availability conditions. Therefore, for this experiment, we defined the stress of exercise objectively, operationally, and independently of energy availability as everything associated with exercise except its energy cost.

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In all these characteristics, except age of menarche, the exercising women were indistinguishable (P > 0.05) from the nonexercising women in whom we had investigated the effects of dietary energy restriction (16). Those women had achieved menarche 1 yr later (13.2 ± 0.2 yr; P < 0.02).

Experimental protocol. To test the energy availability hypothesis in exercising women, we assayed LH in samples drawn at 10-min intervals over 24 h on days 8, 9, or 10 in the follicular phase of two menstrual cycles separated by at least 2 mo after imposing 30 kcal·kg LBM−1·day−1 of exercise on each subject for 4 days beginning on day 5, 6, or 7 of her menstrual cycle. The mean energy intake for the 7-day period was used as an estimate of habitual energy intake, in units of kilocalories per kilogram of LBM per day.

Nine 18- to 29-yr-old volunteers presented with no current use of medications, including oral contraceptives; no history of heart, liver, or renal disease, diabetes, menstrual, or thyroid disorders; and at least 3 mo of documented menstrual cycles 26–32 days in length, between 15 and 30% body fat; and habitual energy intakes between 35 and 55 kcal·kg LBM−1·day−1 based on their 7-day diet records; with maximal aerobic capacities < 42 ml·kg body weight−1·min−1; and a treadmill test for the measurement of aerobic capacity. Aerobic capacity was measured by means of a modified Balke treadmill test as previously described (14). Volunteers kept prospective diet records for seven consecutive days by methods that have also been described (16).

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shown previously that low energy availability induces low menstral cycle. By a similar experimental protocol, we had altered our calculation of the controlled dietary energy intake for the last five exercising subjects in the deprived condition and for the last seven subjects in the balanced condition in this experiment to the designed energy availability plus net exercise energy expenditure (I = A + EEE).

Thus, the first four exercising women in the deprived condition in this experiment (and all the exercising women in our previous experiments) received a low energy availability of 15 kcal·kg LBM−1·day−1, whereas the last five exercising women in this experiment received the intended 10 kcal·kg LBM−1·day−1 like the nonexercising, dietarily restricted women who have been studied in this laboratory. Both of these restricted energy availabilities were well below the threshold energy availability for the maintenance of normal thyroid metabolism that we had identified previously in exercising women [25–30 kcal·kg LBM−1·day−1 by our corrected calculation, and 20–25 kcal·kg LBM−1·day−1 as we had calculated and reported previously (15)]. Similarly, two of the first four exercising women received a balanced energy availability of 50 instead of 45 kcal·kg LBM−1·day−1. Of course, those balanced energy availability treatments were even farther above the threshold energy availability for maintenance of normal thyroid metabolism.

Blood sampling. Beginning 2 days before treatment, blood was sampled on three consecutive days at 0800. Subjects reported to the laboratory after fasting since midnight the previous evening. To control for postural plasma volume shifts, subjects sat for 15 min before blood sampling. After blood was allowed to clot and was spun, the serum was pipetted, stored, and later assayed for T3, insulin-like growth factor-1 (IGF-I), and insulin. In addition, an extra aliquot of blood drawn at 0800 during the 24 h of frequent sampling was processed and stored in the same ways and assayed for the same hormones.

The women were admitted to the General Clinical Research Center (GCRC) of The Ohio State University at 1530 on day 4 of the treatment, and an intravenous catheter was inserted in the forearm at 1600. Blood was drawn every 10 min from 1700 to the next day at 1700. Serum LH was measured in all samples; serum cortisol and follicle-stimulating hormone (FSH) were measured at 30-min and 60-min intervals respectively; and estradiol (E2) was measured at 6-h intervals. Growth hormone (GH) was measured in a pooled sample collected at 10-min intervals throughout the 24-h period. Plasma glucose concentrations were determined in the final four subjects at 30-min intervals by processing a 0.5-ml aliquot of blood immediately after the sample was drawn. Blood samples collected in the GCRC and processed as serum were allowed to clot and were then stored in a refrigerator overnight until they were processed by centrifugation. The resulting serum samples were then aliquoted and stored at −20°C until they were assayed.

The total blood loss from blood sampling was 420 ml in each of the energy availability treatments.

On the evening the subjects were admitted to the GCRC, meals (Ensure) were administered at 1800 and 2100 during the 45 kcal·kg LBM−1·day−1 dietary treatment but only at 1800 during the 10 kcal·kg LBM−1·day−1 treatment. On the following day, meals were administered at 0900 and 1200. Lights were turned on at 2200 and turned back on at 0700. Subjects were observed throughout the day and night by the investigator who was performing blood sampling. Subjects were not allowed to nap during the day, and while lights were out, sleep onset and offset of subjects were recorded.

Assays. LH, FSH, and GH were assayed by two-site monoclonal immunoradiometric kits (Nichols Institute, San Juan Capistrano, CA). LH and FSH standards were calibrated.
against the World Health Organization (WHO) First International Reference (1st IRP 68/40) and the WHO Second International Reference (2nd IRP 78/549), respectively. GH standards were calibrated to the National Institutes of Health reference preparation NIAMD-hGH-RP-1. IGF-I was assayed by a radioimmunoassay (RIA) kit developed by Nichols Institute. An acid ethanol precipitation extraction step was used when performing this assay. Cortisol, insulin, E₂, and T₃ were assayed by means of RIA kits (Coat-a-Count; Diagnostics Products, Los Angeles, CA). For all hormones except LH, interassay variation was avoided by running all samples from each subject in a single assay. The intra-assay coefficients of variation (at specific concentrations) for these other hormones were (in %) 5.4 FSH (5.6 IU/l); 4.5 GH (1.9 µg/l); 5.3 E₂ (180 pM); 4.7 IGF-I (310 ng/ml); 4.2 T₃ (1.8 nM); 7.8 insulin (44 pM); and 4.8 cortisol (310 nM). The large number of LH measurements in each subject could not be performed in a single assay. Therefore, all the samples from each treatment were analyzed in two separate assay runs for each subject. The intra- and interassay coefficients of variation for LH (at a specific concentration) were 4.2 and 7.4% (4.0 IU/l), respectively. Glucose was analyzed enzymatically (YSI model 23L; Yellow Springs Instruments, Yellow Springs, OH) by assaying all samples from each frequent blood sampling session in a separate run. The intra- and interrun coefficients of variation for glucose were 2.8 and 3.4% (4.4 mM), respectively. LH, FSH, E₂, T3, IGF-I, cortisol, and glucose were assayed as the mean of duplicate determinations. T₃, GH, and insulin were assayed as the mean of triplicates.

Data analysis. For each subject, T₃, IGF-I, and insulin concentrations measured on the morning of the two pretreatment days and on the morning of the first treatment day, before beginning treatment, were averaged as the estimate of her baseline T₃, IGF-I, and insulin concentrations. Hematocrit was measured in the daily samples, the plasma volume shifts were calculated (29), and hormone concentrations were adjusted for daily variations in plasma volume. These baseline estimates were subtracted from the same subject’s T₃, IGF-I, and insulin concentrations on the morning after the fourth treatment day as the estimate of her individual metabolic hormone response to the treatment. The difference between these responses to the balanced and deprived energy availability treatments was calculated as the effect of energy availability.

The 24-h transverse means were calculated for LH, FSH, E₂, cortisol, and glucose during the day of frequent sampling. Transverse means for plasma glucose and serum cortisol were also calculated during the feeding phase (0900-2200) and the fasting phase (2200-0900) of the day. LH pulse frequency and amplitude were determined by Cluster analysis, as in our previous investigation of the effects of dietary energy restriction that we had previously reported (16). We then used unpaired, single-sided t-tests to detect and to quantify the independent effects of the stress of exercise on LH pulsatility and metabolic hormones by comparing data from the exercising and nonexercising women (16) under similar balanced and deprived energy availability conditions.

In the absence of consistent a priori information about the signs of exercise stress effects on 24-h mean LH, FSH, and E₂, these parameters were compared by unpaired, two-sided t-tests.

The number of observations in this experiment provided at least a 90% probability of finding differences of 1.1 SD in the tests of the energy availability hypothesis, differences of 1.6 SD in the tests of the exercise stress hypothesis, and differences of 1.8 SD in the comparisons of the magnitudes of the effects of low energy availability caused by exercise energy expenditure and by dietary restriction to be significant at the 0.05 level (7).

RESULTS

Treatments. Table 2 quantifies the experimental power of the diet and exercise regimens. By design, the exercise regimens in the balanced and low energy availability treatments were virtually identical. The controlled submaximal aerobic workloads (%maximal O₂ consumption), the submaximal heart rates, the RPE, the total durations of the daily exercise bouts, and the controlled total energy expenditures (E) during the exercise bouts were indistinguishable. By contrast and also by design, the controlled dietary energy intakes (I) in the balanced and low energy availability treatments were extremely different (P < 10⁻⁷). Despite these differences in dietary energy intake, the subjects complied with the instructions of the investigators to maintain similar 24-h energy expenditure (24EE) as estimated by the physical activity monitors. Net exercise energy expenditure in excess of habitual energy expenditure was also indistinguishable, as intended, in the two energy availability treatments. Thus we achieved an extreme contrast in energy availabilities (P < 10⁻⁸) and estimated 24-h energy balances (P < 10⁻⁶) between the treatments.

There was a noteworthy difference between the exercise treatments, however. The proportion of the exercise energy expenditure derived from carbohydrate oxidation in the deprived energy availability condition was reduced to 49 from 73% in the balanced energy availability condition. This alteration in skeletal muscle fuel selection conserved 253 kcal/day or 63 g/day of carbohydrate in the deprived energy availability treatment.

Because we altered our algorithm for calculating controlled dietary energy intake after the first four exercising women had been studied (see METHODS), the energy availability in the exercising women were marginally greater than energy availabilities in the nonexercising women studied previously (16) in both the balanced (ΔA = 2.7 ± 0.6 kcal·kg LBM⁻¹·day⁻¹; P = 0.06) and deprived (ΔA = 2.8 ± 0.6 kcal·kg LBM⁻¹·day⁻¹; P = 0.04) energy availability treat-
Table 2. Experimental treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Balanced</th>
<th>Restricted</th>
<th>Difference: Restricted - Balanced</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO_{2\text{max}} %, RPE</td>
<td>71.0 ± 0.6</td>
<td>70.6 ± 0.4</td>
<td>-0.3 ± 0.6</td>
<td>0.60</td>
</tr>
<tr>
<td>HR_{\text{max}} %, Duration</td>
<td>84.3 ± 1.2</td>
<td>85.1 ± 1.1</td>
<td>0.8 ± 1.3</td>
<td>0.60</td>
</tr>
<tr>
<td>Carbohydrate oxidation, kcal/day</td>
<td>15.9 ± 0.5</td>
<td>15.9 ± 0.5</td>
<td>0.0 ± 0.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Carbohydrate kcal/kg LBM -1. day^{-1}</td>
<td>179 ± 5</td>
<td>183 ± 3</td>
<td>4 ± 4</td>
<td>0.32</td>
</tr>
<tr>
<td>Controlled energy expenditure (E)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/day</td>
<td>793 ± 44</td>
<td>540 ± 20</td>
<td>253 ± 31</td>
<td>&lt;10^{-4}</td>
</tr>
<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>1,380 ± 70</td>
<td>1,390 ± 40</td>
<td>-20 ± 30</td>
<td>0.62</td>
</tr>
<tr>
<td>Controlled dietary energy intake (I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/day</td>
<td>3.180 ± 110</td>
<td>1.680 ± 50</td>
<td>-1.500 ± 80</td>
<td>&lt;10^{-7}</td>
</tr>
<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>69.9 ± 1.1</td>
<td>36.7 ± 1.0</td>
<td>-33.2 ± 1.1</td>
<td>&lt;10^{-9}</td>
</tr>
<tr>
<td>24-h Energy expenditure (24EE)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>kcal/day</td>
<td>3.120 ± 90</td>
<td>3.080 ± 90</td>
<td>-40 ± 50</td>
<td>0.40</td>
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<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>68.8 ± 0.7</td>
<td>67.0 ± 0.8</td>
<td>-1.8 ± 0.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Exercise energy expenditure (EEE)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>kcal/day</td>
<td>1.090 ± 60</td>
<td>1.100 ± 40</td>
<td>10 ± 40</td>
<td>0.70</td>
</tr>
<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>23.9 ± 0.8</td>
<td>24.0 ± 0.8</td>
<td>0.1 ± 0.8</td>
<td>0.90</td>
</tr>
<tr>
<td>Energy availability (A - i - EEE)</td>
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<tr>
<td>kcal/day</td>
<td>2.090 ± 50</td>
<td>580 ± 50</td>
<td>-1.510 ± 60</td>
<td>&lt;10^{-8}</td>
</tr>
<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>46.0 ± 0.8</td>
<td>12.7 ± 1.1</td>
<td>-33.0 ± 0.9</td>
<td>&lt;10^{-9}</td>
</tr>
<tr>
<td>24-h Energy balance (EB - i - 24EE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/day</td>
<td>60 ± 40</td>
<td>-1.400 ± 80</td>
<td>-1.460 ± 80</td>
<td>&lt;10^{-6}</td>
</tr>
<tr>
<td>kcal/kg LBM -1. day^{-1}</td>
<td>1.1 ± 0.9</td>
<td>-30.3 ± 1.3</td>
<td>-31.4 ± 1.2</td>
<td>&lt;10^{-8}</td>
</tr>
</tbody>
</table>

| P          |
| 0.2 > 0.2  |<10^{-7}|

Values are means ± SE. Differences are paired within individuals. Exercise energy expenditure (EEE) was defined as controlled energy expenditure during exercise (E = 30 kcal·kg LBM -1·day^{-1}) minus energy expenditure estimated by physical activity monitors during the exercise time period on another day, 1 kcal = 4.182 kJ. HR_{max}, maximal heart rate; RPE, rating of perceived exertion.

ments. The estimated 24-h energy balances (EB) of the exercising women, however, were not significantly different from those in the nonexercising women in either the balanced (ΔEB = 1.9 ± 0.8 kcal·kg LBM -1·day^{-1}; P > 0.3) or the deprived energy availability treatment (ΔEB = 2.9 ± 0.8 kcal·kg LBM -1·day^{-1}; P = 0.10).

Effects of low energy availability. The weight (Wt) of the exercising women was unchanged by the balanced energy availability treatment (ΔWt = 0.1 ± 0.2 kg; P > 0.6), but they lost 1.7 ± 0.2 kg (P < 0.0001) or 2.8% of body weight during the low energy availability treatment. Table 3 shows the effects of low energy availability caused by exercise energy expenditure on T_3, GH, IGF-I, insulin, and cortisol. T_3 was reduced by 18%, IGF-I by 26%, and insulin by 34% compared with baseline levels in the normal range. These suppressive effects were similar to those of dietary energy restriction on T_3 (P > 0.3) and insulin (P = 0.4), but the effect on IGF-I was blunted by 50% in the exercising compared with the dietarily restricted women (P < 0.04).

Meanwhile, low energy availability caused by exercise energy expenditure raised the 24-h pooled GH concentration by 26% and the 24-h mean serum cortisol concentration by 11% compared with baseline values in the normal range. The increase in the 24-h mean serum cortisol concentration was caused by a selective elevation of cortisol levels during the fasting phase of the day (2200–0900). Figure 2 shows the mean 24-h cortisol profile of the exercising women during the balanced energy availability treatment and the effect of low energy availability on that profile. Low energy availability raised night time cortisol levels earlier than usual and raised them higher than usual before waking. The effects of low energy availability on 24-h mean serum cortisol concentration were similar in exercising and nonexercising women (P > 0.9), but the stimulatory effect of low energy availability on GH was blunted by 50% in the exercising women compared with that in the nonexercising, dietarily restricted women (not previously reported, P = 0.03).

Figure 3 shows the mean of the plasma glucose profiles measured in the final four exercising subjects during the 24-h frequent blood sampling protocol after the balanced energy availability treatment, along with the suppressive effect of low energy availability on that profile. This effect is quantified in Table 3. Under balanced energy availability conditions, plasma glucose levels declined to a lower (P = 0.04) stable level between 2200 and 0900, when breakfast was served, compared with elevated and highly fluctuating levels between 0900 and 2200, 4 h after the evening meal. Low energy availability reduced plasma glucose levels similarly (P > 0.9) during both of these fasting (P = 0.03) and feeding (P = 0.05) phases of the day and over the entire 24 h (P = 0.03). Because we did not determine the effects of dietary restriction on plasma glucose concentrations, we could not compare the correspond-

Table 3. Effects of energy availability on metabolic hormones and plasma glucose in exercising women

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Baseline Concentration</th>
<th>Treatment Effect: Restricted - Balanced</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_3, nM</td>
<td>1.7 ± 0.07</td>
<td>-0.3 ± 0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>GH, µg/l</td>
<td>2.3 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>376 ± 25</td>
<td>-99 ± 30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Insulin, µM</td>
<td>70 ± 9</td>
<td>-24 ± 6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h mean, nM</td>
<td>190 ± 20</td>
<td>20 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>0900–2200, nM</td>
<td>170 ± 20</td>
<td>10 ± 10</td>
<td>0.30</td>
</tr>
<tr>
<td>2200–0900, nM</td>
<td>210 ± 20</td>
<td>30 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h mean, nM</td>
<td>5.08 ± 0.22</td>
<td>-0.44 ± 0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>0900–2200, nM</td>
<td>5.48 ± 0.37</td>
<td>-0.45 ± 0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>2200–0900, nM</td>
<td>4.56 ± 0.07</td>
<td>-0.44 ± 0.15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Treatment effects are paired differences within individuals. T_3, 3', 5'-triiodothyronine; GH, growth hormone; IGF-I, insulin-like growth factor-I.
The suppressive effect of low energy availability on LH pulse frequency during the waking hours was 57% smaller in exercising women than it had been in nonexercising women \( (P = 0.02) \), but not during sleep \( (P > 0.3) \).

Table 5 summarizes the effects of low energy availability caused by exercise energy expenditure on LH pulse amplitude over the 24-h period. Because the LH pulse amplitudes in the subsamples receiving deprived energy availabilities of 10 and 15 kcal·kg LBM\(^{-1}\)·day\(^{-1}\) and balanced energy availabilities of 45 and 50 kcal·kg LBM\(^{-1}\)·day\(^{-1}\) were also indistinguishable \( (P > 0.6 \text{ and } > 0.7, \text{ respectively}) \), these data from the entire sample were also analyzed as a single group. Low energy availability caused by exercise energy expenditure increased 24-h LH pulse amplitude by 36%, a similar increase \( (P > 0.5) \) to what had been induced by the same low energy availability through dietary restriction in nonexercising women \( (16) \).

Table 5 also shows how the effects of low energy availability caused by exercise expenditure on LH pulse amplitude were distributed between the waking and sleeping hours. LH pulse amplitude increased significantly during sleep after both energy...
availability treatments and by amounts similar to the increases that had occurred in nonexercising women (both P > 0.1). Low energy availability caused by exercise energy expenditure augmented LH pulse amplitude during the waking as well as the sleeping hours, also by amounts similar to those that had been caused by dietary energy restriction (P > 0.3 and > 0.1, respectively).

Low energy availability caused by exercise energy expenditure had no effect on the 24-h transverse mean of LH (Δ = 0.2 ± 0.4 IU/L; P > 0.5) or E2 (Δ = −19 ± 45 pM; P > 0.6), but it did raise the 24-h mean FSH level (from 4.4 ± 0.3 IU/L by Δ = 0.7 ± 0.3 IU/L; P = 0.04).

Table 4. Effects of energy availability and sleep on LH pulse frequency in exercising women

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>Waking</th>
<th>Sleeping</th>
<th>Difference: Waking – Sleeping</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced energy availability</td>
<td>18.9 ± 0.3</td>
<td>20.4 ± 0.7</td>
<td>16.0 ± 1.3</td>
<td>4.4 ± 1.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Restricted energy availability</td>
<td>17.1 ± 0.6</td>
<td>18.1 ± 0.8</td>
<td>15.4 ± 1.5</td>
<td>2.8 ± 1.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Treatment effect: balanced restricted</td>
<td>1.8 ± 0.4</td>
<td>2.3 ± 0.6</td>
<td>0.6 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.002</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Results are expressed in units of pulses/24 h, and results during waking and sleeping are normalized to 24 h. Differences and treatment effects are paired differences within individuals. Statistical tests were single-sided by design, LH, luteinizing hormone.

Effects of the stress of exercise. Baseline concentrations of metabolic hormones were similar in the exercising and nonexercising women (all P > 0.2). Exercise stress did not lower T3 (P > 0.9), insulin (P > 0.7), or IGF-I (P > 0.1) levels in the balanced or the low (all P > 0.7) energy availability treatment. Nor did the stress of exercise raise GH (P > 0.7) or cortisol (P > 0.9) levels in either the balanced or the low (both P > 0.9) energy availability treatment.

Exercise stress did not suppress 24-h LH pulse frequency under either the balanced energy availability condition (18.9 ± 0.4 vs. 19.0 ± 1.5 pulses/24 h, exercising vs. nonexercising, respectively; P > 0.4) or the deprived energy availability condition (17.1 ± 0.6 vs. 16.1 ± 1.7 pulses/24 h, exercising vs. nonexercising, respectively; P > 0.9). Nor did the stress of exercise suppress FSH pulse frequency during either waking or sleeping hours under either energy-balanced or energy-deprived conditions (all P > 0.4).

The stress of exercise did not increase 24-h LH pulse amplitude under either the balanced energy availability condition (1.4 ± 0.1 vs. 1.9 ± 0.2 IU/L, exercising vs. nonexercising, respectively; P > 0.9) or the deprived energy availability condition (1.9 ± 0.1 vs. 2.5 ± 0.3 IU/L, respectively; P > 0.9). Nor did the stress of exercise increase LH pulse amplitude during either the waking or sleeping hours under energy-balanced or energy-deprived conditions (all P > 0.7).

The stress of exercise had no effect on 24-hour LH transverse mean during either balanced (P > 0.2) or deprived (P > 0.4) energy availability, on E2 under either energy availability (P > 0.8 and > 0.3, respectively), or on the 24-h FSH transverse mean during balanced energy availability (P > 0.2). The 24-h FSH transverse mean was elevated by exercise, however, during low energy availability (P = 0.003).

DISCUSSION

The experimental treatments that we administered successfully achieved the objective of exposing the two groups of subjects to extreme, independent contrasts in energy availability and exercise stress, as we operationally defined those terms. The results of this experiment show that 4 days of intense exercise have no disruptive
effect on LH pulsatility apart from the impact of its energy cost on energy availability and that exercise energy expenditure disturbs LH pulsatility less than the equivalent amount of dietary energy restriction.

In this experiment, the balanced energy availabilities administered to the exercising and nonexercising groups were both well above the threshold of energy availability (25–30 kcal·kg LBM⁻¹·day⁻¹) at which energy availability effects on thyroid metabolism abruptly occur (15), and the deprived energy availabilities were both well below this threshold. The small differences in energy availability between the groups were not greater than the day-to-day variation in the subjects’ habitual dietary energy intake. Therefore we doubt that these small group differences in energy availability treatments can account for either the absence of exercise stress effects or the differences in energy availability effects that we observed between the groups.

Stress of exercise. The statistical power of the cross-sectional comparisons by which we tested the exercise stress hypothesis was smaller than those of the repeated-measures comparisons by which we tested the energy availability hypothesis. Nevertheless, we could not attribute our inability to detect disruptive effects of exercise stress on LH pulsatility to limited statistical power, because the LH pulse frequencies in our exercising women were virtually identical to and, if anything, higher than those in our nonexercising women in the balanced and deprived energy availability conditions.

Neither could we attribute our inability to detect a disruptive effect of exercise stress on LH pulsatility to limited experimental power, i.e., to our exercise treatment having been insufficient to reveal a stress effect, because the exercise performed by the habitually sedentary women in this experiment (a total of 1,400 kcal/day at 70% of aerobic capacity) was comparable to running more than 15 miles a day, a workload exceeding the training regimen of all but the most extreme ultramarathon competitors. If the stress of exercise had a disruptive effect on LH pulsatility, we would have found it.

In previous experiments that induced reproductive disorders by exercise training, the stress of exercise was confounded with low energy availability, because dietary energy intake was not supplemented to compensate for the energy cost of the exercise administered (e.g., Refs. 4 and 24). Other stressors thought to interfere with reproductive function also impair energy availability. These stressors include surgery, injuries, burns, and infection (which affect energy availability physiologically) and melancholic depression (which impairs energy availability psychologically and behaviorally through reduced appetite, decreased emphasis on feeding, and sustained anorexia) (5, 22). Research is needed to investigate whether these stressors, like exercise, affect LH pulsatility only through their impact on energy availability.

Much animal research indicates that stress disturbs GnRH neurons by pathways involving corticotropin-releasing hormone, via endogenous opioid and pro-opiomelanocortin-derived peptides, or by dynorphin, biogenic amines, or increased cortisol negative feedback (see Refs. 5 and 22 for reviews). Nevertheless, the neuroanatomical organization and the physiological mechanism of the inhibition of GnRH neurons are still speculative. In general, however, animal experiments demonstrating a suppressive effect of various stressors on LH pulsatility have induced extreme activations of the HPA axis, raising cortisol by several hundred percent. In contrast, exercise raised cortisol levels by only 10% in this experiment, and cortisol levels are elevated only 20–40% in amenorrheic athletes (13, 19) and hypothalamic amenorrheic patients (1).

The strongest evidence of the involvement of the HPA axis in hypothalamic amenorrhea is the 24% suppression of mildly elevated cortisol levels, the 35% increase of LH pulse frequency, and 85% increase in LH pulse amplitude resulting from administration of the corticotropin-releasing hormone antagonist alprazolam to anorexia nervosa patients who had recovered body weight but not menstrual function (9). This evidence says nothing, of course, about whether the HPA axis in these women had been activated by stress, as assumed by the investigators, or by low energy availability or glucose availability.

Energy availability. In this experiment, the effects of low energy availability caused by exercise energy expenditure on LH pulsatility were accompanied by reductions in T₃, insulin, and IGF-I and by increases in cortisol and GH that would be expected under energy-deprived conditions. All these hormones affect reproductive tissues and mobilize stored metabolic fuels (30). Previously, we had observed similar effects of dietary energy restriction on these hormones, except that cortisol levels had not been significantly increased (16). Although an effect of energy availability on cortisol levels was detected in one case and not in the other (perhaps because of differences in statistical power or random variation between small samples), these two cases did not differ from one another (P > 0.9). If this effect does exist, it is small (~10%).

Amenorrheic athletes display low levels of plasma glucose (13), serum T₃ (e.g., 18), and insulin (13), a low ratio of IGF-I to IGF-binding protein-I (13), and increased levels of GH (13) and cortisol (13, 19). Low serum T₃ levels have also been observed repeatedly in anorexia nervosa patients (23), women with dietary amenorrhea (28), and functional hypothalamic amenorrhea (1). Insulin (23) and IGF-I (6) levels are also reported to be low in anorexia nervosa. Circulating IGF-I levels decline with nutritional deprivation (20) and are restored in anorexia nervosa patients with refeeding (6). Slightly elevated cortisol levels are also observed in some (1), but not all (23), nonathletic women with hypothalamic amenorrhea, including anorexia nervosa patients (9). It is particularly noteworthy, therefore, that carbohydrate administration during prolonged exercise prevents the usual exercise-induced rise in cortisol levels in both rats (26) and men (27).
In this experiment, LH pulse frequency was significantly reduced and LH pulse amplitude was significantly increased by 4 days of extreme exercise energy expenditure without any concurrent reduction in the subjects’ habitual dietary energy intake. This finding suggests that athletes may suffer disruptions of reproductive function without practicing restrictive eating behaviors. In this regard, our results extend a previous report that LH pulsatility is suppressed by the combination of strenuous exercise and caloric restriction (32). We have shown that caloric restriction is not necessary for LH pulsatility to be suppressed in exercising women. Exercise energy expenditure alone can reduce energy availability enough to suppress LH pulsatility.

As in our previous experiments (14, 15), the exercising women in this experiment reported that they were satisfied with the amount of food they consumed during the low energy availability treatment and that they had to force themselves to consume all the food they were administered in the balanced energy availability treatment. Thus, hunger may be an insensitive indicator of the energy needs of physically active women, just as thirst is an insensitive indicator of water needs during prolonged exercise (see Ref. 8 for review). Athletes may need to eat by discipline without hunger to prevent reproductive disorders while training, just as they drink by discipline without thirst to prevent dehydration during a long race.

The disruptive effects of low energy availability caused by exercise energy expenditure in this experiment were smaller than those of dietary energy restriction that we had induced previously (16), which had been similar to the alterations in LH pulsatility observed in regularly menstruating athletes with luteal suppression (19). The effects in the exercising women in this experiment were smaller yet than the disruption observed in amenorrheic athletes (19). Those regularly menstruating and amenorrheic athletes had reportedly consumed diets similar to those of sedentary women despite their extremely high levels of physical activity (700 kcal/day), and they had shown endocrine signs of energy deficiency (18). Of course, the larger disruptions of LH pulsatility and ovarian function in athletes might derive from their chronic maintenance of low energy availability habits. Short-term, low energy availability protocols such as ours appear to alter LH pulsatility without affecting ovarian function (21), but we do not know what happens if such extreme dietary and exercise habits or more moderate energy-deficiency behaviors are sustained for periods of weeks, months, and years.

Women in the follicular phase display reduced LH pulse frequencies, with larger pulse amplitudes during sleep than during waking hours (19). By controlling for potential confounding by the darkness and time of day that usually accompany sleep, previous investigators have concluded that this effect is caused by sleep itself (10). However, they did not control for the fast that accompanies sleep, during which gluoregulatory hormones display alterations characteristic of energy deficiency as they stabilize plasma glucose at a reduced level.

Our finding that low energy availability caused by exercise expenditure reduced LH pulse frequency only during the waking (i.e., feeding) hours differs only slightly from our previous finding that low energy availability caused by dietary restriction reduced LH pulse frequency primarily during the waking hours (16). Similarly, our finding that low energy availability caused by exercise energy expenditure increased LH pulse amplitude during both waking and sleeping hours differs by no degree from our previous finding that low energy availability caused by dietary energy restriction increased LH pulse amplitude primarily during sleeping hours. All these findings resemble observations in women with hypothalamic amenorrhea who display a slower LH pulsatile rhythm while awake and larger LH pulse amplitudes while asleep than do regularly menstruating women in the early follicular phase (12).

Glucose availability. Although our experimental disruption of LH pulsatility by reducing energy availability is consistent with the hypothesis that LH pulsatility in women depends on energy availability, we are troubled by the smaller magnitude of this effect in exercising women (1.8 ± 0.4 pulses/24 h) than in the nonexercising women whose dietary energy intake we had restricted by a similar amount (4.4 ± 1.2 pulses/24 h) (16). This seems inconsistent with an implicit assumption of the energy availability hypothesis: that an increase in energy expenditure and a similar reduction in energy intake would have similar effects on LH pulsatility.

Considerable research suggests that in other mammals GnRH neuron activity and LH pulsatility are regulated by brain glucose availability via two separate mechanisms involving the area postrema in the caudal brain stem and the vagus nerve (30, 31). Plasma glucose levels are 10% lower in amenorrheic athletes than in regularly menstruating athletes and sedentary women (13).

The adult female human brain oxidizes ~80 g of glucose each day at a continuous rate, and this must be provided daily by dietary carbohydrate, because the brain’s rate of energy expenditure can deplete liver glycogen stores in <1 day (3). Moderate exercise oxidizes that much glucose in an hour. Routine respiratory-gas analysis during exercise in this experiment revealed that skeletal muscle derived much less energy from carbohydrate oxidation in the deprived energy availability treatment than in the balanced energy availability treatment (49 vs. 73%). This alteration in fuel selection conserved ~70% of the brain’s daily glucose requirement. By contrast, low energy availability caused by dietary energy restriction reduced carbohydrate intake by 77%. This observation leads us to speculate that exercise energy expenditure may compromise brain glucose availability less than the corresponding amount of dietary energy restriction.

In conclusion, our results suggest that prolonged exercise has no disruptive effect on LH pulsatility in women apart from the impact of its energy cost on energy availability or glucose availability, and that LH pulsatility is disturbed less by exercise energy expendi-
turer than by dietary energy restriction. More short-term experiments are needed to resolve whether LH pulsatility depends on energy availability or on glucose availability, and prolonged experiments are needed to confirm that short-term effects on LH pulsatility are predictive of chronic effects on ovarian function. Dietary intervention experiments are also needed to determine whether amenorrheic athletes can prevent or reverse menstrual disorders by dietary reform without moderating their exercise regimen and whether menstrual disorders are more responsive to dietary supplementation than to moderation of exercise. Finally, clinical studies are needed to learn what combinations of dietary and exercise interventions are most effective and best accepted by amenorrheic athletes.

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