Slow restoration of LH pulsatility by refeeding in energetically disrupted women

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Loucks, Anne B., and Mark Verdun. Slow restoration of LH pulsatility by refeeding in energetically disrupted women. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1218–R1226, 1998.—In other energy-restricted mammals, a single large meal restores luteinizing hormone (LH) pulsatility within a few hours. To determine whether this is so in women, we measured LH pulsatility during the 5th day of low energy availability [dietary energy intake – exercise energy expenditure = 10 kcal·kg lean body mass (LBM)−1·day−1] and during a 6th day of aggressive refeeding (90 kcal·kg LBM−1·day−1) in 15 meals providing 4,100 kcal for an energy availability of 75 kcal·kg LBM−1·day−1. Low energy availability raised β-hydroxybutyrate 1,000% (P < 0.001) and reduced plasma glucose 15% (P < 0.01), insulin 63% (P < 0.001), and triiodothyronine 22% (P < 0.005). In five of eight subjects, low energy availability also unambiguously suppressed LH pulse frequency 57% to 8.2 ± 1.5 pulses/24 h (P < 10−5) and raised LH pulse amplitude 94% to 3.1 ± 0.3 IU/L (P < 10−4), levels below the 5th and above the 95th percentile, respectively, in energy-balanced women. Aggressive refeeding restored β-hydroxybutyrate, glucose, and insulin, but not triiodothyronine. In the five women with unambiguously disrupted LH pulsatility, aggressive refeeding had no effect on LH pulse amplitude (P > 0.9) and raised LH pulse frequency only slightly (2.4 ± 0.6 pulses/24 h, P = 0.04) and not above the fifth percentile. This striking contrast between women and other mammals may be another clue to the unidentified mechanism mediating the effect of energy availability on LH pulsatility.

energy availability; nutrition; reproduction; metabolic hormones

THE RESUMPTION OF NORMAL feeding after periods of undernutrition has been reported to restore luteinizing hormone (LH) pulsatility quickly in several mammalian species. A single ad libitum meal has been reported to stimulate LH pulses within only a few hours in food-restricted female rats (2, 3), ewes (5), gilts (1), and male rhesus monkeys (20). Such observations have been interpreted to imply that the physiological signals produced by a single large meal are sufficient to activate gonadotropin-releasing hormone (GnRH) neurons (23).

In previous research we had disrupted LH pulsatility in women by extreme dietary energy restriction (12) and by extreme exercise energy expenditure (16). In so doing, we demonstrated that the LH pulsatility in exercising women depends on energy availability, that is, on the difference between dietary energy intake and exercise energy expenditure, and not on exercise stress. Thus the dietary energy intake that supports normal LH pulsatility in sedentary women fails to do so in exercising women; the apparent suppression of LH pulsatility by exercise can be prevented by dietary supplementation, and exercise has no suppressive effect on LH pulsatility beyond the impact of its energy cost on energy availability. From these observations, we concluded that the mechanism regulating LH pulsatility integrates information about energy intake and energy expenditure.

We suspected that the restoration of LH pulsatility by refeeding might be considerably slower in energetically disrupted women than in other mammals, because the human brain requires so much more energy than does the brain of any other mammal. The adult human brain requires 20% of basal metabolic energy compared with only 2% for most species and 8% for primates (6), and the brain competes against all other tissues of the body for this energy. We reasoned that in humans a single meal might not be enough to activate GnRH neurons.

Indeed, in pilot studies we were unable to observe any effect of refeeding an energetically balanced diet on LH pulsatility within 24 h. Therefore, to more stringently test our hypothesis that the kinetics of the dependence of LH pulsatility on energy availability are uniquely slow in humans, we aggressively overfed energetically disrupted women with an energy availability much greater than energy balance.

MATERIALS AND METHODS

Subjects

Healthy, young, regularly menstruating, habitually sedentary, nonobese, nonsmoking women with no recent history of dieting, weight loss, or aerobic training were recruited from the university and surrounding community. All received a detailed verbal and written description of the study and signed an informed consent document. The experimental protocol was approved by the Institutional Review Boards of Ohio University and The Ohio State University. Before being admitted to the study, volunteers underwent an extensive screening procedure, including written medical, menstrual, dietary, and athletic histories, a physical examination, a 12-lead resting electrocardiogram, a prospective dietary record, determination of body composition by hydrostatic weighing (12), 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 (10). Volunteers kept prospective diet records for seven consecutive days. For this purpose, they were provided with and instructed on the use of dietary scales to weigh all dry food, liquid-measuring cups to measure fluids, and measuring
spoons to quantify smaller portions. The completed dietary records were reviewed for clarity with each subject. Software (Nutritionist IV, San Bruno, CA) was used to calculate the energy content of the diets. 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 lean body mass (LBM) per day.

Eight volunteers 18–28 yr of age presented with no current use of medications including oral contraceptives, no history of heart, liver, or renal disease, diabetes, menstrual or thyroid disorders, ≥3 mo of documented menstrual cycles 26–32 days long, between 15 and 30% body fat, habitual energy intakes between 35 and 55 kcal·kg LBM−1·day−1 based on their 7-day diet records, maximal aerobic capacities <44 ml·kg body wt−1·min−1, and a record of <60 min of habitual aerobic activity per week for the previous 3 mo. Table 1 provides demographic information about the eight young women who completed the experiment and about a subset of five women whose LH pulsatility was unambiguously disrupted by the low energy availability treatment that we administered.

To determine the effect of refeeding on LH pulsatility in this experiment, we compared repeated measurements of LH pulsatility in successive 24-h periods under low and unusually high energy availability conditions. We referenced these pulsatility to measurements of LH pulsatility in successive 24-h periods under low and unusualy high energy availability treatment that we administered. The total duration of the experiment was 202 hours. Exercise energy expenditure (nominally 15 kcal·kg LBM−1·day−1) was set to energy availability to 10 kcal/kg LBM. On day 6, subjects were aggressively refed nominally 90 kcal·kg LBM−1·day−1 for an energy availability of 75 kcal·kg LBM−1·day−1.

Experimental Protocol

To test the hypothesis that refeeding restores normal LH pulsatility in women within 24 h, we assayed LH in blood samples drawn at 10-min intervals for 48 h: first for 24 h on the 5th day of low energy availability treatments and then for 24 h after aggressive refeeding. The 48 h of frequent sampling began on day 8, 9, or 10 of the follicular phase of the menstrual cycle.

Figure 1 illustrates the experimental design. In a sedentary lifestyle, energy balance is achieved by means of an exercise (E) to set energy availability (A = I − E). To impose a low energy availability, we administered a combination of dietary energy restriction (nominally 25 kcal·kg LBM−1·day−1) and energy expenditure during exercise (nominally 15 kcal·kg LBM−1·day−1) to reduce energy availability to 10 kcal·kg LBM−1·day−1. During the aggressive refeeding, we administered a combination of dietary energy supplementation (nominally 90 kcal·kg LBM−1·day−1) in the form of 15 meals providing −4,100 kcal and energy expenditure during exercise (nominally 15 kcal·kg LBM−1·day−1) to set energy availability at 90 − 15 = 75 kcal·kg LBM−1·day−1 (see Dietary energy intake and Exercise energy expenditure for clarification of how these nominal amounts were adjusted to account for habitual energy expenditure during exercise sessions).

Experimental treatments were applied to each woman separately, according to the timing of her individual menstrual cycle. Each subject’s prospectively collected menstrual records were used to predict the onset of her next menses and a tentative schedule for treatments and data collection. That schedule was confirmed at the onset of the next menses. Treatments were applied and data were collected 7 days/wk.

The experimental protocol began on the second, third, or fourth day of each subject’s menstrual cycle and spanned a total of 9 calendar days, including 3 baseline days (days 1–3), 120 h of low energy availability treatments (spanning calendar days 3–8), and 25 h of refeeding (spanning calendar days 8–9). Fasting morning blood samples were drawn at 0800 on the baseline days. Treatments began with the substitution of the controlled diet for the subjects’ habitual diet at 1400 on day 3. Daily exercise treatments began on the morning of day 4. The first 24 h of frequent blood sampling began at 1400 on day 7. Aggressive refeeding began at 1400 on day 8, and the second 24 h of frequent blood sampling began at 1800, after a 4-h sampling interval during which the last exercise treatment was administered. The total duration of the experiment from 0800 on day 1 to 1800 on day 9 was 202 hours.

Frequent blood sampling was performed in the General Clinical Research Center (GCRC) at The Ohio State University Hospital. In the GCRC, lights were turned out at 2300 and turned back on at 0700. Subjects were observed throughout the day and night by the investigator sampling blood. Subjects were not allowed to nap during the day, and their sleep onset and offset were recorded while lights were out.

Exercise energy expenditure. Energy expenditure during exercise was controlled and verified by indirect calorimetry.

Table 1. Demographic characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 8</th>
<th>n = 5</th>
</tr>
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<tbody>
<tr>
<td>Calendar age, yr</td>
<td>21.3 ± 1.1</td>
<td>21.6 ± 1.6</td>
</tr>
<tr>
<td>Age of menarche, yr</td>
<td>13.1 ± 0.4</td>
<td>13.3 ± 0.6</td>
</tr>
<tr>
<td>Gynecological age, yr</td>
<td>7.8 ± 1.0</td>
<td>7.9 ± 1.6</td>
</tr>
<tr>
<td>Menstrual cycle length, days</td>
<td>29.7 ± 0.7</td>
<td>27.6 ± 0.9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>167.2 ± 2.1</td>
<td>165.2 ± 2.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>58.2 ± 3.9</td>
<td>58.5 ± 2.9</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>25.6 ± 1.1</td>
<td>24.4 ± 1.0</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>46.3 ± 1.3</td>
<td>44.8 ± 1.4</td>
</tr>
<tr>
<td>VO2max, ml O2·kg body wt−1·min−1</td>
<td>38.1 ± 1.2</td>
<td>39.3 ± 1.4</td>
</tr>
<tr>
<td>Habitual dietary energy intake kcal/day</td>
<td>2,080 ± 150</td>
<td>1,860 ± 170</td>
</tr>
<tr>
<td>kcal·kg LBM−1·day−1</td>
<td>44.2 ± 2.5</td>
<td>41.4 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. 8 subjects completed experiment; in 5 of these women, low energy availability suppressed LH pulse frequency to below 5th percentile in 18 energy-balanced women. LBM, lean body mass; VO2max, maximal O2 uptake.
All exercise was performed by walking under continuous supervision on a treadmill ergometer in a sequence of 30-min bouts at 70% of each woman’s aerobic capacity interrupted by 10-min rest periods. On days 4–6 of the experimental protocol, exercise was performed on a treadmill in a laboratory at Ohio University. Heart rate and Borg scores of perceived exertion were recorded at minutes 4 and 5 of each exercise bout. Exercise was performed in the GCRC on days 7 and 8, shortly before each 24-h period of frequent sampling; from 1030 to 1230 on day 7 and from 1430 to 1630 on day 8.

Throughout the experiment, each subject wore a physical activity monitor (Caltrac, Hemokinetics, Madison, WI) during all her waking hours, except while bathing, to estimate her 24-h energy expenditure. These instruments use an accelerometer to detect motion and a microprocessor to estimate energy expenditure from the accelerometer signal and from programmed information about the subject’s gender, age, weight, and height. Throughout her waking hours on the baseline days, each subject recorded her displayed integrated energy expenditure at 2-h intervals on preprinted forms. At the same time each morning, a laboratory staff member recorded the 24-h energy expenditure and rezeroed the instrument.

Exercise energy expenditure in excess of habitual energy expenditure was calculated as the total energy expenditure during exercise (E = 15 kcal·kg LBM⁻¹·day⁻¹ measured by indirect calorimetry) minus the energy that would have been expended if the subjects had gone about their usual activities. Estimates of each subject's habitual waking energy expenditure during the exercise time period were obtained from the physical activity monitor records on the baseline days. For most subjects, exercise energy expenditure in excess of habitual energy expenditure was ~12 kcal·kg LBM⁻¹·day⁻¹.

Dietary energy intake. During the low energy availability treatment, a liquid clinical dietary product (Ensure, Ross Laboratories, Columbus, OH) was used to set dietary energy intake. Estimates of each subject’s habitual waking energy expenditure during the exercise time period were obtained from the physical activity monitor records on the baseline days. For most subjects, exercise energy expenditure in excess of habitual energy expenditure was measured by 12 kcal·kg LBM⁻¹·day⁻¹.

Dietary energy intake. During the low energy availability treatment, a liquid clinical dietary product (Ensure, Ross Laboratories, Columbus, OH) was used to set dietary energy intake. Estimates of each subject’s habitual waking energy expenditure during the exercise time period were obtained from the physical activity monitor records on the baseline days. For most subjects, exercise energy expenditure in excess of habitual energy expenditure was measured by 12 kcal·kg LBM⁻¹·day⁻¹.

During the first 24 h of frequent sampling, subjects drank 10 kcal/kg LBM of Ensure at 1300 and 10 kcal/kg LBM at 1800. Compliance was checked by monitoring the urinary ketone acetoacetic acid daily with dip sticks (Multistix, Miles, Elkhart, IN).

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In pilot studies, such aggressive refeeding had quickly brought subjects out of the ketogenic induced by low energy availability, but the subjects had become ketogenic again during sleep. Therefore, we woke the subjects in this experiment at 0300 and fed them an additional meal in an effort to prevent them from becoming ketogenic again at any time during the second 24 h of frequent blood sampling.

Blood Sampling

After fasting since midnight the evening before, subjects reported to the laboratory on days 1–3 of the experimental protocol for baseline blood samples at 0800. To control for postural plasma volume shifts, subjects sat for 15 min before blood sampling. After blood had been allowed to clot and had been spun, the serum was pipetted, stored, and later assayed for β-hydroxybutyrate, estradiol (E2), insulin, and triiodothyronine (T3). Plasma glucose concentrations were determined by processing an aliquot of plasma in an EDTA-treated tube immediately after the sample was drawn. Treatment effects on the fasting levels of these hormones and metabolic substrates were determined by comparing these baseline levels with levels measured in blood samples drawn at 0800 on days 8 and 9 of the experimental protocol, which were processed and stored in the same way.

After the morning blood sample had been drawn on day 7 of the experimental protocol, subjects were driven from Ohio University to the GCRC where they were admitted at 1000. At 1255 an intravenous catheter was inserted in the forearm. Starting at 1400, blood samples were drawn every 10 min for 2 h. The second 24 h of frequent blood sampling began at 1800 on day 8, after that day’s exercise session.

Serum LH was measured in all samples. E2 was measured at 6-h intervals. Plasma glucose concentrations were determined at 30-min intervals by processing a 0.5-ml aliquot of blood immediately after the sample was drawn. During refeeding, additional measurements of β-hydroxybutyrate were made in samples drawn at 1900, 2100, 2300, 0100, 0400, and 0700, and additional measurements of T3 were made in samples drawn at 1800 on days 7 and 9. Blood samples collected in the GCRC and processed as serum were allowed to clot and 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.

Assays

LH was assayed by a two-site monoclonal immunoradiometric kit (Nichols Institute, San Juan Capistrano, CA). The sensitivity of this assay was 0.1 IU/L. LH standards were calibrated against the World Health Organization First International Reference (1st IRP 68/40). Insulin, E2, and T3 were assayed by means of RIA kits (Coat-a-Count, Diagnostics Products, Los Angeles, CA). For all hormones except LH and glucose, interassay variance was avoided by running all samples from each subject in a single assay. The intra-assay variance of variation (at specific concentrations) for these other hormones were 5.6% for E2 (190 pmol/l), 4.0% for T3 (2.1 nmol/l), and 7.0% for insulin (52 pmol/l). 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 3.0 and 4.1% (3.7 IU/L), respectively. Glucose was analyzed enzymatically (model 23L, Yel-
low Springs Instrument, 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 2.4 and 3.5% (4.2 mmol/l), respectively. β-Hydroxybutyrate was also analyzed enzymatically (Sigma Chemical, St. Louis, MO). The intra-assay coefficient of variation for β-hydroxybutyrate at 0.7 mmol/l was 8%. All hormones and substrates were assayed as the mean of duplicate determinations.

### Data Analysis

For each subject, E2, glucose, β-hydroxybutyrate, T3, and insulin concentrations measured at 0800 on baseline days were averaged. Hematocrit was measured in the daily samples, the plasma volume shifts were calculated (25), and hormone concentrations were adjusted for daily variations in plasma volume. These baseline estimates were subtracted from the same subject's glucose, β-hydroxybutyrate, T3, and insulin concentrations on days 8 and 9 to estimate her metabolic responses to low energy availability and refeeding.

Twenty-four-hour transverse means were calculated for LH, E2, and glucose during each day of frequent sampling. LH pulse frequency and amplitude were determined by cluster analysis as in our previous investigation of the effects of dietary energy restriction on LH pulsatility: for peak detection, the algorithm was adjusted to require an increase corresponding to a t statistic of 2.0 for the peak upstroke and downstroke, with peak and nadir test cluster sizes of 1 and 2 points, respectively (12).

### Statistical Analysis

We expected effects of low energy availability on LH and other substrates and hormones to be in the same directions that we had observed previously (12, 16), and we expected the effects of refeeding to be in the opposite directions. Therefore, to confirm the expected effects of low energy availability on metabolic substrates and hormones, we used single-sided paired t-tests to compare measurements made at 0800 on day 8 with the mean of the baseline measurements. To confirm the expected effects of low energy availability on LH, we used single-sided two-sample t-tests to compare data collected in the first 24 h of frequent sampling with data from the reference group of 18 energy-balanced women.

To determine the effect of aggressive refeeding on metabolic substrates and hormones and LH pulsatility, we used single-sided paired t-tests to detect reversals of the low energy availability effects during the second 24 h of frequent sampling. The various metabolic substrates and hormones were examined at selected physiologically relevant times. For example, refeeding effects were sought in all these parameters on awakening after the nighttime fast. Effects were also sought in 24-h mean plasma glucose, in β-hydroxybutyrate at several times before, during, and after the nighttime fast, and in T3 at 1800.

The number of observations in this experiment provided a 90% probability of finding differences of 1.3 standard deviations in two-sample tests confirming the effects of low energy availability and differences of 0.9 standard deviations in single-sample tests detecting the effects of low energy availability and aggressive refeeding to be significant at the 0.05 level (4).

### RESULTS

#### Exercise Treatments

During the low energy availability treatments, the subjects' rate of energy expenditure was controlled at 71.1 ± 0.3% of maximal O2 uptake. At this workload, subjects exercised at 82 ± 1% of their maximum heart rate and rated their perceived exertion at 16 ± 0.5 in the range of 6–20 on the Borg scale of perceived exertion. The total duration of the daily exercise treatments was 93 ± 2 min. Exercise energy expenditure in excess of habitual energy expenditure was 11.8 ± 0.2 kcal·kg LBM−1·day−1.

#### Energy Availability

Dietary energy intakes were 21.7 ± 0.1 and 89.0 ± 1.3 kcal·kg LBM−1·day−1 during the low energy availability and aggressive refeeding treatments, respectively. The dietary and exercise treatments achieved energy availabilities of 9.9 ± 0.2 and 77.2 ± 1.3 kcal·kg LBM−1·day−1 during the first and second 24-h periods of frequent sampling, respectively.

#### Effects on Metabolic Substrates and Hormones

By the morning of the day that they were admitted to the GCRC, the eight women who completed the experiment displayed classic signs of energy deficiency. Their weight had declined by 2.4 ± 0.2 kg (P < 0.0001), i.e., by 3.8% of their initial body weight. Table 2 summarizes the effects of low energy availability on fasting plasma glucose, β-hydroxybutyrate, insulin, and T3 levels. Low energy availability suppressed fasting plasma glucose levels 15% (P < 0.01) and raised fasting β-hydroxybutyrate levels 1,000% (P < 0.001). Insulin levels fell 63% (P < 0.001), and T3 levels fell 22% (P < 0.005).

Figure 2 shows the mean of the plasma glucose profiles during the first 24 h of frequent blood sampling and the restorative effect of aggressive refeeding on that profile. Table 2 summarizes the effects of aggressive refeeding on fasting plasma glucose, β-hydroxybutyrate, insulin, and T3 levels in the eight women studied. Aggressive refeeding raised 24-h mean plasma glucose levels 28% (P < 0.01) and restored 0800 fasting glucose levels 28% (P < 0.01). Table 2 summarizes the effects of aggressive refeeding on metabolic substrates and hormones in women who completed experiment.

<table>
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<th>Substrate/Hormone</th>
<th>Baseline</th>
<th>Restricted</th>
<th>Refed</th>
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<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800</td>
<td>4.7 ± 0.2</td>
<td>3.9 ± 0.1a</td>
<td>4.9 ± 0.3a,e</td>
</tr>
<tr>
<td>24-h mean</td>
<td>4.8 ± 0.1</td>
<td>6.1 ± 0.2d</td>
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</tr>
<tr>
<td>β-Hydroxybutyrate, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800</td>
<td>0.1 ± 0.04</td>
<td>2.0 ± 0.4p</td>
<td></td>
</tr>
<tr>
<td>1900</td>
<td>0.4 ± 0.1d</td>
<td>0.4 ± 0.1d</td>
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</tr>
<tr>
<td>2100</td>
<td>0.1 ± 0.1d</td>
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<td></td>
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<tr>
<td>2300</td>
<td>0.2 ± 0.1d</td>
<td></td>
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<tr>
<td>0100</td>
<td>0.2 ± 0.1d</td>
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<td>0400</td>
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<tr>
<td>0700</td>
<td>0.2 ± 0.1d</td>
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<tr>
<td>Insulin, pmol/l</td>
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<tr>
<td>0800</td>
<td>91 ± 14</td>
<td>33 ± 5p</td>
<td>73 ± 13e</td>
</tr>
<tr>
<td>T3, nmol/l</td>
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<tr>
<td>0800</td>
<td>1.7 ± 0.1</td>
<td>1.3 ± 0.1b</td>
<td>1.4 ± 0.1b,e</td>
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<tr>
<td>1800</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
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</table>

Values are means ± SE for 8 women. T3, triiodothyronine. Statistical tests are by repeated measures: *P < 0.05, **P < 0.01 vs. baseline; ***P < 0.01 vs. restricted. *Sample was drawn at 0700.
plasma glucose levels ($P > 0.4$). β-Hydroxybutyrate was restored to baseline levels ($P = 0.9$) 4 h after hourly feedings began, and it remained in that range. Despite the meal at 0300, a slight increase in β-hydroxybutyrate levels was detected ($P < 0.05$) by 0700, but this was due to a reduction in variance at that time. Insulin was restored to within the baseline range ($P > 0.05$) by 0700 on day 9, but T$_3$ was not restored by 1800 on day 9 ($P < 0.01$).

Effects on LH Pulsatility

Figure 3 shows a representative LH pulse profile from 1 of the 18 women we had studied previously while their energy availability was controlled at 45 kcal·kg LBM$^{-1}$·day$^{-1}$. The mean and standard deviation (SD) of the LH pulse frequencies in this group as a whole were 18.9 and 2.5 pulses/24 h, respectively. The mean and SD of their LH pulse amplitudes were 1.6 and 0.5 IU/l, respectively. Thus we would expect 95% of such women to display LH pulse frequencies greater than the mean $- 1.74 \times SD = 14.6$ pulses/24 h and LH pulse amplitudes less than the mean $+ 1.74 \times SD = 2.5$ IU/l.

Figure 4 shows a representative pair of LH pulse profiles from one of the three women whose suppression of LH pulsatility by low energy availability in this experiment was ambiguous. Pulse profiles are shown during the first 24 h of frequent sampling during restricted energy availability and during the second 24 h of aggressive refeeding. The LH pulse frequencies of these women under restricted energy availability conditions were $> 14.6$ pulses/24 h and in two cases even $> 18.9$ pulses/24 h. These women might have displayed LH pulse frequencies considerably above average under energy-balanced conditions, but we could not confidently conclude from their low energy availability data alone that they had responded to the low energy availability treatment or that their data during refeeding would be a fair test of the effect of refeeding on energetically suppressed LH pulsatility. Therefore, we tested the refeeding hypothesis with and without the data from these women in the data set.

Figure 5 shows a representative pair of LH pulse profiles from one of the five subjects whose suppression of LH pulsatility by low energy availability in this experiment was unambiguous. Again, pulse profiles are shown during the first 24 h of frequent sampling during restricted energy availability and during the second 24
h of aggressive refeeding. In these women, low energy availability suppressed LH pulse frequency 57% to 8.2 ± 1.5 pulses/24 h (P < 10^{-4}). This was 4.3 ± SD below the mean (18.9 pulses/24 h) and well below the fifth percentile (14.6 pulses/24 h) of LH pulse frequency in our energy-balanced reference group. Low energy availability also increased LH pulse amplitude in these women by 94% to 3.1 ± 0.3 IU/l, 3.0 ± SD above the mean (1.6 ± 0.5 IU/l, P < 10^{-4}), and well above the 95th percentile (2.5 IU/l) of LH pulse amplitude in the reference group. The effects of energy availability on metabolic substrates and hormones in the five women whose LH pulsatility was unambiguously suppressed were similar to those in the group as a whole (Table 3).

Figure 6 illustrates the effects of low energy availability and aggressive refeeding on LH pulsatility in all eight women in this experiment and in the five women whose LH pulsatility was unambiguously suppressed by low energy availability. Among the five women whose LH pulsatility had been unambiguously suppressed by low energy availability, aggressive refeeding raised LH pulse frequency (P = 0.04), but by only 2.4 ± 1.0 pulses/24 h. As a result, their LH pulse frequencies on the day of aggressive refeeding remained far below the fifth percentile (14.6 pulses/24 h) of LH pulse frequency in the energy-balanced reference group. Aggressive refeeding did not raise LH pulse frequency above the fifth percentile in any of these women. The unambiguously elevated LH pulse amplitudes were completely unaffected (Δ = 0.0 ± 0.4 IU/l, P > 0.90) by aggressive refeeding. The 24-h LH transverse mean was also unaffected (Δ = 0.8 ± 0.4 IU/l, P = 0.12). Results were similar when all eight women were included in the analysis. Aggressive refeeding pushed the group as a whole only to, but not past, the 5th and 95th percentiles of LH pulse frequency and amplitude, respectively.

At baseline, E2 levels at 0800 were 120 ± 10 pmol/l. These levels were unaffected by low energy availability.
(150 ± 30 pmol/l, P = 0.16) at 0800 on day 8 of the experiment. Aggressive refeeding also had no effect (P = 0.18) on the 24-h mean $E_2$ level during the final 24 h of frequent blood sampling (100 ± 20 pmol/l) compared with the first 24 h (110 ± 20 pmol/l).

**DISCUSSION**

This experiment was designed to test whether refeeding restores LH pulsatility in energetically disrupted women within 24 h, as it has been reported to do in other mammalian species. By design, the low energy availability (9.9 kcal·kg LBM$^{-1}$·day$^{-1}$) used to disrupt LH pulsatility in this experiment was much less than the energy availability threshold (20–25 kcal·kg LBM$^{-1}$·day$^{-1}$) below which $T_3$ production is suppressed in exercising women (11). As we had observed previously (12, 16), this low energy availability unambiguously disrupted LH pulsatility in five of the subjects. Also by design, the extremely high energy availability administered during aggressive refeeding (77 kcal·kg LBM$^{-1}$·day$^{-1}$) was much greater than the balanced energy availability (45 kcal·kg LBM$^{-1}$·day$^{-1}$) at which we had previously characterized normal LH pulsatility in other habitually sedentary women (12, 16). Nevertheless, 24 h of such aggressive refeeding had very little effect on LH pulsatility in these women, in sharp contrast to the reported restoration of LH pulsing within hours in other mammals.

**Effects of Low Energy Availability**

The hypothesis that reproductive function depends on energy availability is supported by an extensive literature on the bioenergetics of reproduction in mammalian species ranging from rodents to humans (for reviews see Refs. 26 and 27) as well as by clinical observations. Athletic women consume less energy than would be expected for their activity level (8, 15, 18), and amenorrheic athletes show endocrine signs of chronic energy deficiency (8, 14, 15). In these respects, amenorrheic athletes are similar to women with dietary amenorrhea (24), anorexia nervosa (21), and functional hypothalamic amenorrhea (7). Finally, in fasting monkeys (22) and exercising women (16, 29), LH pulsatility is disrupted by low energy availability rather than by stress.

In this experiment, low energy availability caused by a combination of moderate dietary restriction and moderate exercise energy expenditure suppressed plasma glucose, insulin, and $T_3$ levels and raised $\beta$-hydroxybutyrate levels as subjects mobilized body fat stores to supplement an inadequate dietary energy intake. Low energy availability also suppressed LH pulse frequency and increased LH pulse amplitude, as had low energy availability caused by extreme dietary restriction alone (12) and extreme exercise expenditure alone (16). We did not measure LH pulsatility under energy-balanced conditions in this experiment, but we have no doubt that low energy availability disrupted LH pulsatility in at least five of our eight subjects, because after 4 days of low energy availability treatments their LH pulse frequency and amplitude were below the 5th and above the 95th percentile of LH pulse frequency and amplitude, respectively, among the energy-balanced women previously measured in this laboratory.

Although we may have disturbed LH pulsatility in our three other subjects, we cannot be sure, because their LH pulsatility during low energy availability was not unusual. Because one would not expect refeeding to restore LH pulsatility that had not been strongly disrupted, we focused our attention on the five women whose LH pulsatility had been unambiguously disrupted to maximize our likelihood of detecting the effect of refeeding.

Before proceeding, however, these three subjects warrant an additional comment. There was a minority of subjects with considerably smaller effects of low energy availability on LH pulsatility in our earlier experiments as well (12, 16). Of course, their presence in the samples reduced the reported mean effects of energy availability on LH pulsatility in those experiments. These experimental results complement observational data on athletes (8, 15) in suggesting that some women may be considerably more robust than others in maintaining normal reproductive function under apparently similar energy-deficient conditions.

**Effects of Aggressive Refeeding**

Hourly refeeding restored baseline $\beta$-hydroxybutyrate levels within 4 h, suggesting that refeeding must have suppressed ketone production well below the ketone oxidation rate almost immediately. Plasma glucose and insulin levels were also quickly restored. By contrast, 15 meals providing ~4,100 kcal were unable to restore $T_3$ to baseline levels within 24 h. In an earlier investigation of short-term energy availability effects on $T_3$ (10), 2 days of low energy availability were required to suppress $T_3$ levels, and these levels remained low for ≥2 days after ad libitum feeding resumed. These observations demonstrate that the kinetics of the thyroid axis in responding to changes in energy availability are much slower than those of the glucoregulatory system.

In this experiment, aggressive refeeding had very little normalizing effect on LH pulsatility in the energetically disrupted women within 24 h. Only a very small increase in LH pulse frequency and no decrease in LH pulse amplitude were detected, so that LH pulse frequency and amplitude remained beyond their 5th and 95th percentiles, respectively.

Two types of experiments have reported effects of refeeding on energetically suppressed LH pulsatility. In one type performed on prepubertal animals, one group is fed a weight maintenance diet while another group receives a diet that supports normal growth, and effects on LH pulsatility and sexual development are observed (2, 3, 5). In such chronically food-restricted animals, LH pulsing is severely and often completely suppressed, and ad libitum refeeding restarts LH pulsing within hours, thereby initiating an accelerated “catch-up” in pubertal development, with the first
ovulation occurring well before body weight increases to the weight at which the first ovulation occurs in normally growing animals.

In the second type of experiment, refeeding follows only a few days of energy restriction (1, 17, 19, 20, 22, 23, 28). After prepubertal gilts had been feed restricted for 7 days, feeding to appetite restored episodic LH secretion within 6 h (1). In male rhesus monkeys the diurnal pattern of LH pulsatility depends on the timing of the daily meal, with LH pulses occurring more frequently in the hours after feeding (17). LH pulsatility is severely suppressed after 1–2 days of fasting, and refeeding restores LH pulsatility within 2 h (20) in proportion to the size of the meal administered (20). Intragastric infusions of mixed nutrients with an energy content equivalent to that of a normal daily meal restore LH pulsatility during continued fasting, indicating that it is the nutritional or metabolic signal, not the psychological stress of fasting, that disrupts LH pulsatility in this model (22). Furthermore, intragastric infusions of dextrose and a carbohydrate-free mixture of casein and lipids were as effective as an intragastric infusion of mixed nutrients in restoring LH pulsatility on the 2nd day of fasting (23). All three infusions increased LH pulse frequency on the 2nd day by 2.3–3 times, even though blood glucose levels remained low after the casein and lipid infusion. The latter observation indicates that LH pulsatility does not depend on blood glucose levels. Nor does it appear to depend on insulin, since insulin suppression fails to prevent the restoration of LH pulsatility by refeeding (28).

To our knowledge, only one other experiment has reported data on the effects of refeeding on energetically suppressed LH pulsatility and metabolic hormones in normally cycling women (19). In that experiment, LH pulsatility was observed for 8 h on the 3rd day of fasting and on the 2nd day of refeeding in the midfollicular phase. Fifty-six hours of fasting reduced LH pulse frequency by 21% and T3 levels by 38%. By the 2nd day of refeeding, LH pulsatility and T3 had returned to normal. Of course, we did not monitor our subjects on the 2nd day of refeeding, but we remain skeptical that they would have returned to normal, because they were so little affected by the first 24 h of such aggressive refeeding and because LH pulse frequency during the last few hours was no higher than that during the first few hours. More refeeding may be required for recovery from the more extreme suppression of LH pulsatility caused by a longer period of energy deficiency in our experimental protocol.

Stress

Because treatments were applied to only a single cohort of women in this experiment, it might be speculated that the commercially prepared liquid meals, the schedule for restriction and refeeding, or the exercise regimen might constitute a stressful disruption of the habitual lifestyle of these subjects and that one or more of these speculative stressors, rather than low energy availability, might have suppressed their LH pulsatility. If low energy availability did not disrupt the LH pulsatility, then refeeding would not be expected to restore it, and this experiment would not be a valid test of our hypothesis.

Our previous research demonstrated convincingly that whatever stress may be associated with the same liquid meals and with exercise, speculative stress did not disrupt LH pulsatility in those experiments (9, 12, 16). From that we infer that these speculative stresses did not suppress LH pulsatility in this experiment. The speculative stress of consuming the same commercially prepared liquid meals for 4 days did not disrupt LH pulsatility when sedentary women consumed these meals in sufficient amount to provide a balanced energy availability of 45 kcal·kg LBM$^{-1}$·day$^{-1}$ (12). Nor did the speculative stress of exercise at 70% of maximal O2 uptake have a suppressive effect on LH pulsatility beyond the impact of the energy cost of that exercise on energy availability (16), for the suppression of LH pulsatility in exercising women was prevented by dietary supplementation (16). Nor did the speculative stress of consuming 70 kcal·kg LBM$^{-1}$·day$^{-1}$ of these same meals for 4 days suppress LH pulsatility (16).

We cannot yet make the same convincing argument from currently available data with respect to the speculative stress of dietary restriction. In our previous investigation of dietary restriction, energy availability and the speculative stress of dietary restriction are confounded (12). These two factors are not necessarily confounded, however, and a future experiment could distinguish their independent effects. We will predict now that when that experiment is performed, the speculative stress of dietary restriction will have no suppressive effect on LH pulsatility beyond the impact of dietary restriction on energy availability.

Nor can we yet make a convincing argument from currently available data that the speculative stress of awakening subjects in the middle of the night has no suppressive effect on LH pulsatility. Although such an effect is a logical possibility, previous research would not lead one to expect it to occur, since LH pulse frequency is faster during hours of wakefulness than during hours of sleep (12, 16). Still, it is logically possible that awakening subjects in the middle of the night might have had a sustained suppressive effect throughout the following day, and our present data cannot rule out this possibility. This speculative stress, too, is susceptible to experimental investigation, however, and again we will predict now that it, too, will have no suppressive effect on LH pulsatility.

Perspectives

The energy availability hypothesis holds that the GnRH pulse generator in the hypothalamus is disrupted by an as yet unidentified signal that dietary energy intake is insufficient for the organism’s current level of energy expenditure. Despite a refeeding protocol much more aggressive than the ad libitum feeding commonly employed in animal experiments, our protocol had little restorative effect on LH pulsatility in our energetically suppressed women. Apparently, this protocol was not sufficient to stimulate GnRH neurons in
women. We speculate that this striking difference from other mammalian species may be related to the equally striking difference between the proportions of basal metabolic energy consumed by the brains of humans and all other mammals. A striking species difference in the kinetics of this mechanism offers additional insights and another avenue of investigation in the effort to identify the mechanism.

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REFERENCES


13. Loucks, A. B., and S. M. Horvath. SLOW RESTORATION OF LH PULSATILITY


