Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women

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Hilton, L. K., and A. B. Loucks. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am. J. Physiol. Endocrinol. Metab. 278: E43–E49, 2000.—Because the effect of exercise on leptin was not established, we controlled energy intake (I) and exercise energy expenditure (E) to distinguish the independent effects of energy availability (A = I − E) and exercise stress (everything associated with exercise except its energy cost) on the diurnal leptin rhythm in healthy young women. In random order, we set A = 45 and 10 kcal·kg lean body mass–1·day–1 for 4 days during the early follicular phase of separate menstrual cycles in sedentary (S, n = 7) and exercising (X, n = 9: E = 30 kcal·kg LBM–1·day–1) women. Low energy availability suppressed the 24-h mean (P < 0.05) and amplitude (P < 10–5), whereas exercise stress did not (both P > 0.2). Suppressions of the 24-h mean (–72 ± 3 vs. –53 ± 3%, P < 0.001) and amplitude (–85 ± 3 vs. –58 ± 6%, P < 0.001) were more extreme in S vs. X than previously reported effects on luteinizing hormone pulsatility and carbohydrate availability. Thus the diurnal rhythm of leptin depends on energy, or carbohydrate, availability, not intake, and exercise has no suppressive effect on the diurnal rhythm of leptin beyond the impact of its energy cost on energy availability.

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luteinizing hormone; pulsatility; reproduction; carbohydrate

AMENORRHEIC and regularly menstruating athletes consume less dietary energy than would be expected for their level of physical activity, and they are similarly lean (23, 27) and display similarly low 24-h mean leptin levels (23). Yet amenorrheic athletes display a more extreme suppression and disorganization of luteinizing hormone (LH) pulsatility (27), as well as lower plasma glucose and insulin levels and a complete suppression of the amplitude of the diurnal rhythm of leptin (23).

Administration of leptin antiserum markedly suppresses LH pulsatility in female rats (7), and the administration of leptin itself prevents the suppression of LH pulsatility induced by fasting in both female rats (32) and male rhesus monkeys (12). Because leptin is secreted by adipocytes and correlates highly with body mass index (BMI), body adiposity, and fat mass (8), leptin was first hypothesized to signal information about fat stores (29). Later, reports that profound fluctuations in leptin occur before changes in body adiposity, in response to fasting (19, 44), dietary restriction (44), refeeding after dietary restriction (15, 19), and overfeeding (20) led to the hypothesis that leptin signals information about dietary energy intake, particularly dietary carbohydrate intake (15).

Little has been established about the effects of exercise on leptin. Others have not resolved whether the stress and/or energy cost of exercise suppresses leptin (14, 34, 35), perhaps because data were not collected in an experimental design that distinguished the effects of stress from those of energy intake and expenditure, or perhaps because leptin was measured in single daily blood samples, neglecting its diurnal rhythm, and perhaps because exercise treatments were not severe enough to reveal an effect.

To determine the independent effects of energy availability (operationally defined as dietary energy intake minus exercise energy expenditure) and exercise stress (operationally defined as everything associated with exercise except its energy cost) on the 24-h mean and amplitude of the diurnal rhythm of leptin, we assayed stored samples from an earlier experiment in which we administered dietary and exercise treatments to regularly menstruating women for 4 days and then sampled blood at 10-min intervals for 24 h to distinguish the independent effects of these factors on LH pulsatility and certain metabolic hormones (26). We then related these new findings to our previously reported results to gain additional insight into the regulation of LH pulsatility in exercising women.

METHODS

Subjects. Young, healthy, habitually sedentary, nonsmoking, normal weight women were recruited from Ohio University and the surrounding community. Before their participation, volunteers received a detailed oral and written description of the screening process and experimental protocol, which was approved by the Institutional Review Boards of Ohio University and The Ohio State University. All volunteers signed an informed consent document.

Subjects were screened as previously described (26, 28) to exclude volunteers with a history of menstrual or thyroid disorders, diabetes, or other known health problems, along with those currently taking any medication, including oral contraceptives. Additional qualification criteria included a dietary energy intake between 35 and 55 kcal·kg of lean body mass (LBM)–1·day–1, a body composition between 18 and 30% body fat, no recent history of dieting or weight loss, a maximal aerobic capacity of <42 ml O2·kg body wt–1·min–1, and habitual aerobic activity of <60 min/wk.

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Design. The independent effects of energy availability and exercise stress were determined by a prospective, $2 \times 2$ (energy availability × exercise stress) experimental design (Fig. 1) (28). Energy availability was defined operationally as dietary energy intake minus exercise energy expenditure. Exercise stress was also defined operationally and independently as everything, physiological and psychological, associated with exercise except its energy cost.

Subjects were assigned to sedentary and exercising groups, and each subject was studied twice, in random order, under balanced (B) and low (L) energy availability conditions. To achieve a balanced energy availability (SB: $A = I - E = 45$ kcal·kg LBM$^{-1}$·day$^{-1}$, where $A$ is availability, $I$ is intake, and $E$ is expenditure) in the sedentary group ($S$, $n = 7$), a dietary energy intake of $I = 45$ kcal·kg LBM$^{-1}$·day$^{-1}$ was administered. The low energy availability (SL: $A = I - E = 10$ kcal·kg LBM$^{-1}$·day$^{-1}$) was achieved by restricting their dietary energy intake to $I = 10$ kcal·kg LBM$^{-1}$·day$^{-1}$.

In the exercising group ($X$, $n = 9$), the low energy availability (XL: $A = I - E = 10$ kcal·kg LBM$^{-1}$·day$^{-1}$) was achieved by administering a dietary energy intake of $I = 40$ kcal·kg LBM$^{-1}$·day$^{-1}$ and an exercise workload of $E = 30$ kcal·kg LBM$^{-1}$·day$^{-1}$. The balanced energy availability condition (XB: $A = I - E = 45$ kcal·kg LBM$^{-1}$·day$^{-1}$) was achieved by raising their dietary energy intake to $I = 75$ kcal·kg LBM$^{-1}$·day$^{-1}$ in compensation for the same exercise workload. Thus effects of low energy availability could be determined by comparing data from groups SL and XL with data measured in groups SB and XB, respectively, and the independent effects of exercise stress could be determined by comparing data from groups SB and XB with data measured in groups SB and SL, respectively.

The groups were similar in habitual daily dietary energy intake, height, weight, body fatness, BMI, LBM, age, and length of the menstrual cycle (Table 1: all $P > 0.05$). Habitual dietary energy intakes were 45.7 ± 2.2 kcal·kg LBM$^{-1}$·day$^{-1}$ in $S$ and $X$, respectively.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Units</th>
<th>Diet</th>
<th>Restricted (S)</th>
<th>Exercise (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yr</td>
<td></td>
<td>7</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Height cm</td>
<td></td>
<td>165.8 ± 1.6</td>
<td>162.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Body weight kg</td>
<td></td>
<td>58.6 ± 2.1</td>
<td>61.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Body fat %</td>
<td></td>
<td>24.6 ± 1.3</td>
<td>26.6 ± 0.8</td>
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</tr>
<tr>
<td>BMI kg/m²</td>
<td></td>
<td>42.8 ± 1.3</td>
<td>45.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>$V_{O_2\text{max}}$ ml O₂·kg body wt$^{-1}$</td>
<td></td>
<td>39.8 ± 1.4</td>
<td>38.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Dietary intake kcal·kg LBM$^{-1}$·day$^{-1}$</td>
<td></td>
<td>45.7 ± 2.2</td>
<td>48.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Menstrual cycle length days</td>
<td></td>
<td>29.9 ± 1.3</td>
<td>28.7 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE; $n$, no. of subjects; LBM, lean body mass; BMI, body mass index; $V_{O_2\text{max}}$, maximal aerobic power.

Protocol. The experimental protocol has been described in detail previously (28). After two baseline days without treatments, treatments were administered for 4 days beginning on days 5, 6, or 7 of the menstrual cycle. To assess 24-h energy expenditure, subjects wore an activity monitor (Caltrac, Hemokinetics, Madison, WI) during all waking hours, except while showering, throughout the experiment. In both groups, 24-h energy expenditures were similar under both energy availability conditions ($P > 0.3$).

The daily exercise treatment consisted of a series of supervised 30-min bouts of walking on a treadmill at 70% of each individual's aerobic capacity, with 10-min rest intervals between bouts until each subject had expended 30 kcal·kg LBM$^{-1}$·day$^{-1}$.

After the baseline days, dietary energy intake was controlled by feeding subjects measured amounts of the liquid dietary product, Ensure (Ross Products Division, Abbott Laboratories, Columbus, OH). Subjects were permitted to drink water ad libitum, but nothing else. Compliance with the energy availability treatments was checked each morning by monitoring urinary acetacetate levels with Multistix dip sticks (Miles, Elkhart, IN).

At 1530 on the 4th day of experimental treatments, subjects were admitted to the General Clinical Research Center (GCRC) at The Ohio State University Hospital. Subjects consumed the balance of that day's controlled diet in meals scheduled at 1800 (B and L) and 2100 (B only). The next day, subjects consumed their entire controlled diets in meals scheduled at 0900 and 1200. No allowance was made for the decreased energy expenditure due to bed rest during the 24 h of frequent sampling.

Blood sampling. In the GCRC, an indwelling venous catheter was inserted in subjects at 1600, and blood was sampled every 10 min for 24 h beginning at 1700. Samples were allowed to clot, stored in a refrigerator overnight, and then were centrifuged, pipetted into storage tubes, and stored at −20°C until they were assayed for leptin.

Assays. Leptin was assayed as the mean of duplicate determinations in samples drawn at 60-min intervals. Assays were made by RIA kits obtained from Linco Research (St. Charles, MO). The intra-assay and interassay coefficients of variation for leptin were 5.3 and 7.5% at 3 ng/ml, 3.4 and 3.7% at 12 ng/ml, and 2.6 and 5.6% at 16 ng/ml, respectively.

Data analysis. For each subject, 24-h mean leptin concentration was calculated, and leptin time series were expressed both as absolute concentrations and as relative concentrations normalized to the 24-h mean for each subject. The
RESULTS

Figure 2 shows the leptin profiles measured during the 24-h frequent blood sampling period. The profiles are presented separately as concentrations and as percentage changes from each individual’s 24-h mean. Profiles for balanced and low energy availability treatments are plotted together for comparison. Table 2 presents summary statistics for the profiles shown in Fig. 2.

When the sedentary women were administered the balanced energy availability treatment, cosinor rhythmometry detected a significant diurnal rhythm in each subject (all P < 0.05). At 18.7 ± 2.3 ng/ml, the acrophase occurred at 0100, and the nadir of 9.5 ± 1.1 ng/ml occurred at ~1100. The 24-h mean and amplitude of the diurnal rhythm were 14.3 ± 1.8 ng/ml and 4.6 ± 0.7 ng/ml, respectively. The amplitude was 32 ± 2% of the 24-h mean. (The 24-h mean was correlated with percent body fat (r = 0.61, P = 0.01), explaining 37% of the variance in the 24-h mean among the subjects.)

After the balanced energy availability treatment, leptin concentrations were substantially higher at the end of the 24-h frequent blood sampling period than at the beginning. This occurred in both the sedentary and exercising women (Δ = 7.8 ± 1.4 ng/ml = 75 ± 18% and Δ = 3.7 ± 1.0 ng/ml = 41 ± 15% of the initial levels, respectively; both P < 0.01), although the increase was approximately twice as great in the sedentary women (P < 0.05). This mismatch between the beginning and end of the 24-h frequent blood sampling period was caused by an excessive rise in leptin during the afternoon on the second day of sampling. After the low energy availability treatment, leptin concentrations

Table 2. Leptin parameters after balanced energy availability treatments and effects of low energy availability

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>Sedentary (S)</th>
<th>Exercise (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Balanced</td>
<td>L effect</td>
</tr>
<tr>
<td>Leptin concentrations</td>
<td>24-H Mean</td>
<td>14.3 ± 1.8</td>
<td>−10.5 ± 1.6†</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>−10.5 ± 1.6</td>
<td>−72 ± 3%‡</td>
</tr>
<tr>
<td></td>
<td>At acrophase</td>
<td>18.7 ± 2.3</td>
<td>−14.4 ± 2.0†</td>
</tr>
<tr>
<td></td>
<td>At nadir</td>
<td>9.5 ± 1.1</td>
<td>−6.5 ± 1.0†</td>
</tr>
<tr>
<td></td>
<td>Amplitude</td>
<td>4.6 ± 0.7</td>
<td>−3.9 ± 0.6‡</td>
</tr>
<tr>
<td></td>
<td>% Change</td>
<td>−85 ± 3‡</td>
<td>−56 ± 6‡</td>
</tr>
</tbody>
</table>

All values are means ± SE. Effects of low energy availability by exercise expenditure less than effects by dietary restriction: *P < 0.05. Effects of low energy availability (L effect = low energy availability values – balanced energy availability values): †P < 0.01, ‡P < 0.001.
were also higher in the sedentary women at the end of the frequent blood sampling period (by 1.2 ± 0.2 ng/ml = 37 ± 6% of the initial level; P ≤ 0.001). This rise had no effect on the change in 24-h mean leptin levels in either group (both P > 0.2), although it slightly reduced the percentage change in 24-h mean leptin levels in the exercising women (−1.5 ± 0.6%, P < 0.05) but not in the sedentary women (P > 0.1).

Exercise stress effects. Comparing exercising women (X) to sedentary women (S) at the same energy availabilities revealed that exercise stress had no suppressive effect on either the 24-h mean (P > 0.2) or the amplitude (P > 0.3) of the diurnal leptin rhythm. As mentioned above, however, the unexpectedly excessive rises in leptin during the afternoon of the 2nd day of sampling were smaller in the exercising than in the sedentary women.

Energy availability effects. Comparing women receiving low energy availability treatments (L) with themselves when they received balanced energy availability treatments revealed that low energy availability strongly suppressed both the 24-h mean (P < 10⁻⁶) and amplitude (P < 10⁻⁵) of the diurnal rhythm of leptin (Fig. 2, Table 2). Low energy availability blunted the amplitude of the leptin rhythm by >10% in all seven sedentary women, and cosinor rhythm analysis was unable to detect a significant rhythm in two of the women (both P < 1.5, P > 0.1). By contrast, the rhythm was maintained in all nine of the exercising women during the low energy availability treatment (all P > 0.5, P < 0.1), and the amplitude was blunted by >10% in only two.

Thus there was an interaction between energy availability and exercise stress on both the 24-h mean and amplitude of the diurnal leptin rhythm (P < 0.02). This interaction was due to the greater suppression of both the 24-h mean (−72 ± 3 vs. −53 ± 3%, P < 0.001) and amplitude (−85 ± 3 vs. −58 ± 6%, P < 0.01) of leptin in the sedentary women by low energy availability. Indeed, when expressed as a percentage of its 24-h mean, the amplitude of the diurnal leptin rhythm was suppressed only in the sedentary women (from 32 ± 2 to 17 ± 2% in the sedentary women, P < 0.01, vs. from 31 ± 2 to 27 ± 3% in the exercising women, P > 0.5).

**DISCUSSION**

The diurnal rhythm of leptin. Under continuous enteral nutrition, the diurnal rhythm of leptin in men mirrors the diurnal rhythm of rectal temperature (38). The amplitude of the rhythm under these conditions is ~9% of the 24-h mean (38). About one-half of this amplitude is truly circadian, and about one-half is attributable to the reduction of metabolic rate during sleep (38). The amplitude increases to >30% of the 24-h mean when an energy-balanced diet is consumed as oral meals within the usual 12-h span (37). This predominant dietary component of the rhythm shifts with the timing of these meals (37). When insulin levels decline to fasting levels a few hours after sleep onset, leptin levels begin to fall rapidly. This fall stops shortly after breakfast, and then leptin rises slowly throughout the afternoon and evening in a pattern that appears to depend on the proportions of daily energy consumed at each meal (37), and in which upward excursions in leptin are temporally associated with meal-mediated peaks in insulin (23).

The diurnal rhythm of leptin displayed by the women in this experiment after the balanced energy availability treatments (Fig. 2) was similar to the rhythms observed in other groups who consumed their diets as oral meals (21, 25, 37, 39). As in some other experiments (21, 25), however, leptin levels were much higher at the end of the 24-h frequent blood sampling period than at the beginning because of an excessive rise in leptin during the afternoon of the 2nd day of our 24-h frequent blood sampling period (Fig. 2), probably as an artifact of our feeding schedule. Throughout the treatment period, we controlled energy availability on a 24-h clock from 0800 to 0800, but the frequent blood sampling period was scheduled from 1700 to 1700. During the frequent blood sampling, we administered the balance of the first day's food between 1700 and midnight, in keeping with the feeding schedule during the treatment period, but we administered the entire next day's food between 0900 and 1200, causing an unusually strong drive for leptin secretion in the afternoon. We observed no excessive rise in leptin during the afternoon in a subsequent study of 18 subjects who received the same energy availabilities at the same times during the 24-h frequent blood sampling period as they did during the preceding treatment period (Loucks, unpublished data). Thus the differences in leptin concentrations at the end of the 24-h blood sampling procedure are attributable to differences in our feeding schedule. This illustrates the acute and profound responsiveness of leptin to feeding and the need to carefully control dietary intake in studies of the effects of other factors on leptin and LH pulsatility.

Exercise stress. Exercise stress had no suppressive effect on either the 24-h mean or amplitude of the diurnal rhythm of leptin. Apparently, suppressive effects of exercise in women consuming 45 kcal·kg LBM⁻¹·day⁻¹ of dietary energy were prevented by supplementing their diets in compensation for their exercise energy expenditure. Thus the only influence of exercise on the 24-h mean and amplitude of leptin occurred via the impact of its energy cost on energy availability.

Previous investigators had reported inconsistent effects of prolonged exercise training (12–64 wk) on leptin (14, 18, 34, 35). In these studies, either dietary energy intake or exercise energy expenditure was uncontrolled, with the result that energy availability was also uncontrolled and confounded with the stress of exercise. These studies also neglected the diurnal rhythm of leptin. Only one previous study (41) has assessed effects of exercise on the 24-h rhythm of leptin, finding the 24-h mean, but not the amplitude, to be suppressed in men when exercise was performed under conditions of energy balance, indicating an effect of exercise stress. That study imposed only a moderate exercise workload (~12 kcal·kg LBM⁻¹·day⁻¹) for only
1 day. Our untrained women performed a workload two and one-half times greater, equivalent to running a half-marathon, every day for 4 days. Thus, if exercise stress were capable of disrupting the 24-h mean and amplitude of the diurnal rhythm of leptin in women, our exercise treatment should have revealed that effect.

It must be admitted, however, that the leptin responses to our unintended overfeeding of the subjects on the 2nd day in the GCRC were smaller in the exercising women than in the sedentary women. Therefore, one might argue that here, at least, we did detect a suppressive effect of exercise stress on leptin. Stress presumably mediation by the adrenal axis, however, and this particular effect might be explainable without invoking the adrenal axis. For if our exercise treatment depleted skeletal muscle glycogen stores, then the subsequent repletion of those stores during overfeeding may have diverted glucose away from adipose tissue. A similar exercise protocol (5 days of running 12.5 miles/day at 80% maximal aerobic capacity) did, indeed, deplete muscle glycogen stores by ~36% in male distance runners consuming an isocaloric diet containing ~530 g CHO/day (17). Therefore, perhaps we saw here not an effect of exercise stress per se, but rather an effect of exercise on the availability of glucose to adipose tissue.

Energy availability. The 72% suppression of 24-h mean leptin caused by 4 days of 78% dietary restriction in this experiment was similar to the 75% decrease in 24-h mean leptin caused by 2 days of fasting (3), and more profound than the 54% decrease caused by fasting for 4 days (13), the 64% decrease caused by a 50% reduction (~1,000 kcal) in dietary energy intake for 4 days (15), and the 61% decrease caused by dietary restriction to 630 kcal/day for 7 days (11). All of these findings are in contrast to the absence of any effect of a 28% negative energy balance for 1 day on the 24-h mean and amplitude of the diurnal rhythm of leptin in exercising men (41). Perhaps energy availability must be reduced by >28% for effects to be detected, or maybe men and women differ in their sensitivity to energy availability. Clearly, however, because the 24-h mean and amplitude of the diurnal rhythm of leptin were suppressed by 53 and 58%, respectively, in our exercising women while they consumed ~1,700 kcal/day of dietary energy, dietary energy intake is not what controls leptin levels. Like other metabolic and reproductive hormones that we have studied in women (26, 28), leptin responds to the difference between intake and expenditure.

Carbohydrate availability. In humans, leptin falls by 50% during dietary restriction before body weight falls by >2%, and most of this weight loss is due to fluid losses secondary to glycogen depletion and proteolysis (15). Furthermore, leptin-secreting mechanisms appear to be “blind” to dietary fat (15). Rather, leptin declines in proportion to reductions in dietary carbohydrate (15). In isolated rat adipocytes, leptin secretion was found to be directly proportional to glucose uptake, and the blockade of glucose uptake inhibited leptin secretion in a dose-response manner (31). Because earlier studies had indicated the importance of glucose in the regulation of reproductive function in mammals (42), we had calculated an observational quantity that we named carbohydrate availability as an index of the availability of glucose to metabolic tissues (28). We defined carbohydrate availability as the controlled dietary carbohydrate intake administered to each subject minus her carbohydrate oxidation during exercise.

Both balanced energy availability treatments in this experiment provided ~1,000 kcal/day of carbohydrate availability, but skeletal muscle altered its fuel selection profoundly in response to the low energy availability treatment, oxidizing less carbohydrate and more fat during exercise (28). As a result, despite identical low energy availabilities, carbohydrate availability was 57% higher in our women whose energy availability was reduced by exercise than in our women whose energy availability was reduced by dietary restriction (385 ± 30 vs. 242 ± 6 kcal/day, P < 0.01) (28). An infusion of 300–400 kcal/day of glucose has been reported to prevent or temporarily reverse the fasting-induced suppression of leptin in humans (4, 13). This range includes the carbohydrate availability in our exercising women and the daily glucose requirement of the central nervous system in adult humans (6). Thus the smaller effects of low energy availability on the diurnal rhythm of leptin in our exercising women may be explained by a greater availability of glucose to adipose tissue.

The intracellular mechanism regulating the apparent dependence of leptin secretion on glucose availability is not yet clear. Mueller et al. (31) concluded that the stimulation of leptin secretion must occur downstream of phosphofructokinase in the metabolic pathway of energy metabolism, because leptin (ob) gene expression and secretion were directly proportional to fructose as well as glucose uptake, and because they were inhibited in a dose-response manner by the inhibition of glycolysis by iodoacetate and sodium fluoride. The inhibition of glycolysis backs up intermediates that feed back to inhibit glucose uptake.

By contrast, Wang et al. (43) found that leptin (ob) gene expression and secretion were stimulated in rats when glycolysis was inhibited by the infusion of fatty acids. In their experience, inhibition of glycolysis increases the flux of glucose through the hexosamine pathway, which synthesizes mucopolysaccharides from fructose 6-phosphate, despite a concurrent inhibition of glucose uptake. Wang et al. concluded that leptin (ob) gene expression and secretion must be regulated in the hexosamine pathway, after they found that leptin (ob) gene expression and secretion were also increased in both adipose and skeletal muscle tissues when rats were infused with either glycosamine or uridine (43). Glucosamine is an intermediate in the hexosamine pathway, and uridine is a substrate in it. Further study is needed to resolve the apparently contrary effects of inhibiting glycolysis by different means.

Associated effects on LH pulsatility. The suppressive effects of low energy availability and the absence of a
The suppressive effect of exercise stress on the 24-h mean and amplitude of the diurnal rhythm of leptin in this experiment closely resemble our previous report of the suppressive effects of low energy availability and the absence of a suppressive effect of exercise stress on LH pulsatility (26, 28). Furthermore, the suppressive effects of low energy availability on LH pulsatility, like those on the diurnal rhythm of leptin, were smaller in our exercising women than in our dietarily restricted women (28). Thus the similar treatment effects on carbohydrate availability, the diurnal rhythm of leptin, and LH pulse frequency in our experiment suggest that LH pulsatility may depend on carbohydrate rather than energy availability, although they do not resolve whether that dependence is mediated or merely modulated by leptin.

Investigators who did not find an effect of fasting on LH pulsatility in women, despite a 75% suppression of pooled 24-h leptin (3), suggested two differences between their experiment and those experiments of investigators who found effects of fasting and low energy availability on LH pulsatility (28, 33) to explain this inconsistency: their subjects were tested during the luteal phase instead of the follicular phase, and their data were analyzed by deconvolution instead of cluster analysis. A third difference is that they began frequent blood sampling when subjects had been fasting for only 32 h. Suppressive effects of low energy availability on LH pulsatility have been detected by cluster analysis in the follicular phase when frequent blood sampling was begun after 60 h of fasting (33) and after 89 h of low energy availability (28). Thus a reasonable explanation for the apparent inconsistency may be that leptin responds more quickly than LH pulsatility to low energy or carbohydrate availability.

Considerable animal research indicates that the apparent dependence of LH pulsatility on energy availability is mediated by leptin (9). Leptin administration has these effects without preventing or restoring fasting-induced reductions in weight, blood glucose, and insulin or the increase in β-hydroxybutyrate (1).

Leptin appears to enter the brain at the median eminence, where the surrounding structures display the highest levels of leptin binding in the brain (2), and to have its effect on gonadotropin-releasing hormone via neuropeptide Y and proopiomelanocortin neurons (1, 12, 24, 30). Leptin may also act on the reproductive system peripherally by modulating a glucose signal to the central nervous system (16, 36). The infusion of 300–400 kcal/day of glucose has been reported to prevent or temporarily reverse the fasting-induced suppression of lepitin in humans (4, 13) and is strikingly similar to the daily glucose requirement of the brain in adult humans (6). Therefore, the smaller effects of low energy availability on LH pulsatility in our exercising women may be explained by a greater availability of glucose to the brain.

Although the experimental evidence that leptin mediates the influence of glucose availability on LH pulsatility is tempting, it must be admitted that it is not consistent with the entire body of clinical observations in anorexia nervosa patients (40), amenorrheic cathletes (23), and women with functional hypothalamic amenorrhea (22). Thus neither clinical observations nor experimental results have yet established whether leptin plays a mediating or modulating role in the hypothesized dependence of reproductive function on glucose availability.

In summary, low energy availability profoundly suppressed the 24-h mean and amplitude of the diurnal rhythm of leptin in this experiment, whereas exercise stress had no such effects. Effects of low energy availability caused by exercise energy expenditure were smaller than those caused by dietary restriction, apparently because skeletal muscle altered its fuel selection during exercise, thereby conserving substantial amounts of glucose. During the second afternoon of frequent blood sampling, an artifactual excessive rise in leptin illustrates the acute and profound responsiveness of leptin to feeding and the need to carefully control dietary intake in studies of the effects of other factors on leptin and LH pulsatility.

We are grateful to H. Brient, Dr. E. Heath, S. J. Robinson, K. M. King, T. Law, Sr., Dr. W. B. Malarkey, D. B. Morrell, J. Petrosini, A. Pfugard, Dr. K. Ragg, M. Rinaldi, J. R. Thuma, and M. Verdun for their important contributions to this research. We also appreciate the extraordinary cooperation of the subjects.

This research was presented at the 79th Annual Endocrine Society Meeting, 1997, Minneapolis, MN (Abstract no. P1–375). It was supported by National Institutes of Health (NIH) Dr. L. Shettam Award 1R55HD-29547–01; the American Heart Association, Ohio Affiliate; the Ohio University College of Osteopathic Medicine; the Ohio University; John Hauk Memorial Research Grant; and, in part, Grant M01 RR00034 from the General Clinical Research Branch, Division of Research Resources, NIH.

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Received 27 April 1999; accepted in final form 30 August 1999.

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