Serum Leptin Concentrations in Response to Acute Exercise in Postmenopausal Women With and Without Hormone Replacement Therapy (44400)

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Abstract. **The purpose of the study was to examine the effects of acute exercise and hormone replacement therapy on serum leptin concentrations in postmenopausal women. Subjects were 15 healthy, postmenopausal women, 8 on hormone replacement therapy (HRT) and 7 not on hormone replacement therapy (NHRT). Group comparisons indicated no significant differences between HRT and NHRT groups with respect to age, height, weight, BMI, sum of skinfolds, or VO_2max, and verified significant differences in estradiol and FSH concentrations. After an overnight fast, each subject completed 30 min of treadmill exercise at ≈80% VO_2max. Over 2 hr and 10 min, baseline, exercise, and recovery blood samples were collected from an intravenous catheter. A control session conducted a month later consisted of the same blood sampling protocol without exercise. Leptin concentrations declined significantly over the course of both the exercise and control sessions, gradually decreasing from baseline levels to −1.54 ± 0.49 ng · ml^{-1} postexercise, and continuing to decline to a low of −2.89 ± 0.59 ng · ml^{-1} at the end of the session. There was no significant difference between groups with respect to this decline. This is the first study to document that diurnal changes in leptin concentrations in postmenopausal women are not altered by acute treadmill exercise or HRT status. The study underscores the need to account for a diurnal reduction in leptin over the course of an exercise trial.**

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Leptin has been identified as an "antiobesity hormone" that may also play a role as a regulator of neuroendocrine function (1). Leptin is secreted primarily from adipose tissue and is thought to act through signal-transducing leptin receptors in the hypothalamus (2). It is likely that leptin exerts its most important effects through the central nervous system. There is much data from rodent models to demonstrate that leptin acts through neuropeptide Y (NPY). NPY cells in the arcuate nucleus contain leptin receptors, and leptin has been found to suppress NPY (1). Leptin receptors have also been identified in the ovary (3) and in a number of other tissues (4). It has been shown that a mutation in the *ob* gene in mice is associated with increased food intake and infertility due to lack of leptin produced by adipocytes and that leptin administration reverses these states (5, 6). It is also known that leptin overrides fasting-induced inhibition of gonadotropin concentrations in both the *ob/ob* and normal mice. Thus, it appears that leptin serves as a metabolic signal that integrates nutritional status and reproduction (3).

It has been suggested that estrogen may act to modulate messages produced by the medial hypothalamus (a site of estradiol receptors) that controls fasting signals. This speculation came about from evidence that after castration of the

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female rodent, there is an increase in food intake and reduced activity of the sympathetic nervous system (4). When estrogens are injected or directly applied to the hypothalamus structures of intact rodents, there is a reduction in food intake.

Women have been shown to have higher leptin levels than men (7, 8). The role of leptin is of particular interest for postmenopausal women, since an increase in body weight and central obesity is common in this population (9). Although our lab has found no difference in resting leptin levels of postmenopausal women taking estrogen compared with women not on estrogen (7), other research utilizing estrogen treatment in ovariectomized women supports the possibility that estrogen regulates leptin production (10). Estrogen receptors have been reported in adipose tissue (11, 12). This, coupled with the evidence that serum leptin concentrations in premenopausal women are higher during the luteal phase than the follicular phase of the menstrual cycle (10), indicates the need for study of postmenopausal women whose estrogen concentrations are determined by hormone replacement therapy.

A few investigators have examined the effects of exercise on leptin concentrations in women. The effects of exercise on leptin concentration is of interest since it has been suggested that if leptin serves as a signal for regulation of energy intake and since exercise is known to alter energy balance, then leptin might serve to mediate the demands of exercise (13). It has been shown that acute exercise suppresses expression of the *ob* gene in rats by 30% (14). In one recent study, training adaptations of middle-aged men and women were compared (8). Although fat mass was not changed after exercise training, leptin levels were reduced in the females but not in the males, suggesting a gender difference for the effect of exercise training on leptin concentrations. A few investigators have examined the acute effects of exercise on leptin concentrations, but no studies have examined acute responses in postmenopausal women. The few studies that have examined exercise responses have not employed a frequent sampling protocol to measure leptin concentrations before, during, and after acute exercise, which is important for discerning any changes across time. Kohrt *et al.* (15) have documented a reduction in resting leptin levels in postmenopausal women following 9 months of aerobic training. Thus, it is not known whether acute exercise affects leptin concentrations in postmenopausal women, nor is it known whether estrogen, which is thought to increase the expression of the leptin gene, affects these responses.

We have examined a group of nonobese (BMI ≈25.0) postmenopausal women to study the effects of hormone replacement therapy (HRT) and exercise on a variety of anterior pituitary and adrenal hormones (16, 17). We have extended these studies to examine the effects of acute exercise and hormone replacement therapy on serum leptin concentrations in a subgroup of these same subjects. Thus, the focus of the study was not on leptin responses in obese women, but rather on normal postmenopausal women. These data are important to determine whether there are exercise-induced alterations in leptin concentrations in postmenopausal women, and whether they are affected by standard estrogen replacement therapy.

Materials and Methods

Subjects. Thirty postmenopausal women volunteered for the study; 15 who met the criteria and were selected provided written consent for participation. Eight of the women were on estrogen replacement (Ogen, Premarin, or Estratest); two of these women were also taking progestin. Seven of the HRT subjects took HRT daily; one subject took HRT on Days 1–25 (this subject had been on HRT for 14 days when tested). The latter subjects were scheduled for the experimental protocol on days when they were taking progestin. Seven women were not taking any hormone replacement (NHRT). Menopause had been surgically induced (removal of the uterus and both ovaries) in three of the women on HRT and in one of the untreated women. All other subjects experienced menopause naturally (cessation of menses for at least 1 year). Mean (± SEM) age was 49.37 (± 3.07) and 51.57 (± 3.54) years for the HRT and NHRT groups, respectively. The differences in estrogen therapy and causes of menopause reflect the postmenopausal population, and the way in which HRT is practiced. Other descriptive characteristics are shown in Table I.

Subjects met the following criteria for participation in the study: (1) postmenopausal status; (2) ability to complete 30 min of moderate treadmill exercise; (3) free from chronic illnesses such as diabetes mellitus, liver or gallbladder disease, coronary heart disease, malignancy, anemia, or renal failure; and (4) not taking any medication that could alter hormone concentrations such as β-blockers, glucocorticoids, diuretics, or other hormonal or hormone-mimetic medications. Subjects were judged to be healthy following a medical history screening and a graded exercise test with 12-lead electrocardiogram. The study was approved by the Southeastern Louisiana University Investigative Review Board

Table I. Descriptive Characteristics of Subjects[a]

Group	Height (cm)	Weight (kg)	BMI (Wt/Ht^2)	Sum of skinfolds (mm)	VO_{2max} (ml/kg/min)
HRT	162.71 ± 2.37	64.71 ± 4.31	24.26 ± 1.03	88.25 ± 9.95	29.37 ± 1.35
NHRT	165.64 ± 1.37	69.22 ± 6.61	25.22 ± 2.31	81.71 ± 13.72	24.95 ± 3.48

[a] Values represent mean ± SEM.

and was completed in accordance with the Declaration of Helsinki.

Pre-Experimental Trial. Subjects completed a pre-experimental session for body composition assessment by measurement of height, weight, and skinfolds. Treadmill maximal oxygen uptake (VO_{2max}) was assessed with an automated VO_2 measurement system. Expiratory air volume was determined with a heated pneumotach (Series 3813, Hans Rudolph, Kansas City, MO) and pressure transducer (VRCD/HC-1, Consentius Technologies, Sandy, UT); expired O_2 and CO_2 were assessed (Ametek S-3 a/1 and CD-4 analyzers, Pittsburgh, PA). Equipment was interfaced (OUS/MC, Consentius Technologies, Sandy, UT) to a personal computer, and values were recorded every 30 sec. Prior to each VO_{2max} determination, the O_2 and CO_2 gas analyzers were calibrated with gases of known composition. The treadmill protocol began at 2 miles/hr, 2% grade, and every 2 min it was increased 0.5 miles/hr, 2% grade until 3.5 miles/hr was reached; thereafter, only treadmill grade was increased by 3% every 2 min until exhaustion. All subjects were determined to have reached VO_{2max} when either the primary criterion of a plateau in VO_2 with an increase in workload was met or two of three secondary criteria were noted: (1) reaching predicted maximum heart rate; (2) respiratory exchange ratio > 1.0; or (3) a rating of perceived exertion (RPE, Borg Scale) of 9 or 10 (18).

Experimental and Control Trials. The subjects reported to the exercise physiology laboratory at 0745 hr following an overnight fast. An intravenous catheter (Travenol, 22 g, 32 mm) was inserted into an antecubital vein, and a normal saline lock was attached. At 0900, 40 min prior to exercise (–40) and at 0910, 10 min prior to exercise (–10), resting blood samples were collected from the catheter while the subject was in a sitting position. For each blood draw, the first 3 ml of blood (with saline from the catheter lock) was withdrawn into a discard tube preceding a 25-ml draw. The catheter was then flushed with physiological saline (3 ml) to maintain patency. Subjects completed 30 min of treadmill exercise at 78.66 ± 1.09% VO_{2max} (HRT) and 79.99 ± 1.44% VO_{2max} (NHRT). Exercise intensity was maintained by adjusting speed and grade of the treadmill. Blood was collected during exercise (after 15 and 30 min; +15, +30). Additional blood draws were taken in a sitting position at 10, 20, 35, 50, 65, and 80 min postexercise (during recovery, R).

A third session served as a control trial and consisted of the same blood sampling time points while the subject rested. The sessions were not randomized. The control trials were scheduled for the third session. Due to the nature of this older population of women, we felt that it was less stressful and threatening for the subjects to be fully aware of the order and details of each testing procedure and that having the subjects complete the exercise session first would help to ensure higher compliance. Moreover, we thought it was wise to perform the more important protocol first.

Blood Analyses. For each blood draw, samples were collected in two 10-ml whole blood tubes for endocrine determinations, a 5-ml EDTA tube for hematocrit and hemoglobin assays, and a 3-ml sodium fluoride/potassium oxalate tube for plasma lactate analysis (colorimetric method, Sigma Chemical, St. Louis, MO). Whole blood was centrifuged and serum aliquotted and frozen (–20°C) for subsequent determination of leptin concentrations. Lactate concentration was used to document the relative degree of anaerobiosis produced by exercise. Hematocrit (microcapillary method) and hemoglobin values (colorimetric method, Sigma Chemical, St. Louis, MO) were used to determine plasma volume change (19) and to correct hormone levels for hemoconcentration shifts that could inherently elevate hormone levels (20). Plasma volume shifts for time periods –10 to +15, +15 to +30, +30 to R10, R10 to R20, R20 to R35, R35 to R50, R50 to R65, and R65 to R80 were as follows: –7.39 ± 2.49%, 4.04 ± 3.42%, 5.34 ± 1.91%, 1.15 ± 2.43%, 1.42 ± 2.70%, 1.99 ± 1.13%, 1.52 ± 1.07%, and –2.34 ± 1.69%, respectively, for the HRT group and –7.27 ± 4.33%, 5.35 ± 2.41%, 3.78 ± 0.90%, 0.04 ± 1.44%, 1.66 ± 1.55%, 3.86 ± 2.07%, –1.96 ± 1.96%, and 0.55 ± 1.35%, respectively, for the NHRT group. Estradiol, FSH, and LH concentrations were determined in baseline samples to verify the reproductive hormone status of the women and provide a complete endocrine profile of each subject.

Lactate concentrations were analyzed spectrophotometrically (Sigma Chemical, St. Louis, MO). Leptin was determined in duplicate with a ^{125}I RIA (LINCO Research, St. Charles, MO). Serum E_2, FSH, and LH concentrations were determined using an automated chemiluminescent assay (IMMULITE; Diagnostic Products Corp., Los Angeles, CA). All of the hormone serum samples from each subject were determined in the same assay to avoid any changes in interassay variability. Intraassay coefficients of variation for leptin, E_2, FSH, and LH concentrations were less than 5.0%. Interassay coefficients of variation for leptin, E_2, FSH, and LH concentrations for the low, middle, and high pools were all <10%, with the exception of the low pool for E_2 (14.51%).

Results

During the exercise trial, lactate concentrations rose from 1.14 ± 0.16 m*M* and 0.82 ± 0.14 m*M* at –40 to peak at 5.78 ± 0.80 m*M* and 4.82 ± 0.79 m*M* at +15 for HRT and NHRT groups, respectively. Independent *t* tests were used to verify that groups were not different in physical characteristics (age, height, weight, BMI, sum of skinfolds) or VO_{2max}, but were different relative to estradiol and FSH concentrations. The latter were one-tailed analyses as we expected E_2 to be higher and FSH to be lower in the HRT group than in the NHRT group. Group means and standard errors for these variables are reported in Tables I and II. The results of these *t* tests confirmed that HRT and NHRT groups were not significantly different for any of the physical characteristics or VO_{2max}. In addition, as expected, rest-

Table II. Baseline (−10) Gonadotropin and Estradiol Concentrations[a]

Group	FSH (mIU · ml^{-1})	LH (mIU · ml^{-1})	E_2 (pg · ml^{-1})
HRT	32.19 ± 6.13[b]	24.76 ± 4.04	83.96 ± 28.34[b]
NHRT	63.49 ± 13.21	27.34 ± 3.16	27.56 ± 4.20

[a] Values represent mean ± SEM.
[b] $P < 0.05$ between groups.

ing E_2 concentrations were significantly higher in the HRT group [t (13) = −1.84, $P < 0.05$], and resting FSH concentrations were significantly lower in the HRT group [t (13) = 2.25, $P < 0.05$].

Leptin concentrations were analyzed using two different approaches. In the first set of analyses, a baseline level was calculated for each participant by averaging her first two leptin concentrations (−40 and −10). Leptin concentrations for each subsequent measure were transformed into change scores by subtracting the baseline level from each value. As shown in Figure 1, leptin levels decreased over the morning for HRT and NHRT groups during both exercise and control sessions, with the greatest decline evident in the HRT group during the exercise session.

These data were analyzed using a 2 × 2 × 9 (trial × group × time) ANOVA with repeated measures on the last factor. A significant time effect was revealed [F (8, 208) = 6.83, $P < 0.0001$], signifying a significant decline in leptin levels over time, gradually decreasing from baseline concentrations to −1.54 ± 0.49 ng/ml at +30, and continuing to decline to a low of − 2.89 ± 0.59 ng/ml at R80. Eta squared was calculated to indicate effect sizes. Approximately 21% the total variance in leptin change scores was explained by this factor. *Post hoc* power analysis indicated a 98% probability that an effect of this magnitude would be detected as statistically significant using the 2 × 2 × 9 repeated measures design. No other main effects nor interactions reached significance.

Second, in order to examine the total leptin response, an integrated area under the leptin response curve (AUC) was calculated for each subject. AUC is a summary value that allows the cumulative effect of a stimulus on an endocrine parameter to be examined (21, 22). These values are calculated using a trapezoidal method after subtracting each subject's baseline hormone concentration. The mean value for concentrations at −10 and −40 for each subject was used as her baseline hormone concentration. Mean AUC values varied considerably among participants, with standard errors ranging from 50.19 to 117.15 ng · min · ml^{-1}. AUC during the control session was similar for HRT and NHRT groups (HRT mean = −174.07 ± 50.19 ng · min · ml^{-1}; NHRT mean = −213.78 ± 53.64 ng · min · ml^{-1}), comparable to that of the NHRT group during exercise (−203.18 ± 117.15 ng · min · ml^{-1}), and noticeably lower for the HRT group during the exercise trial (−02.92 ± 109.59 ng · min · ml^{-1}). Analysis of these values using a 2 × 2 (group × trial) ANOVA, however, yielded no significant main effects nor interactions. Eta squared estimates of the percentage of variance explained by each factor indicated that only 1% was accounted for by the group factor, 6% by the trial factor, 8% by the interaction, and 85% attributable to inter- and intra-subject variability. *Post hoc* power analysis indicated a relatively low probability (17%) that the analysis would detect these small effect sizes as statistically significant.

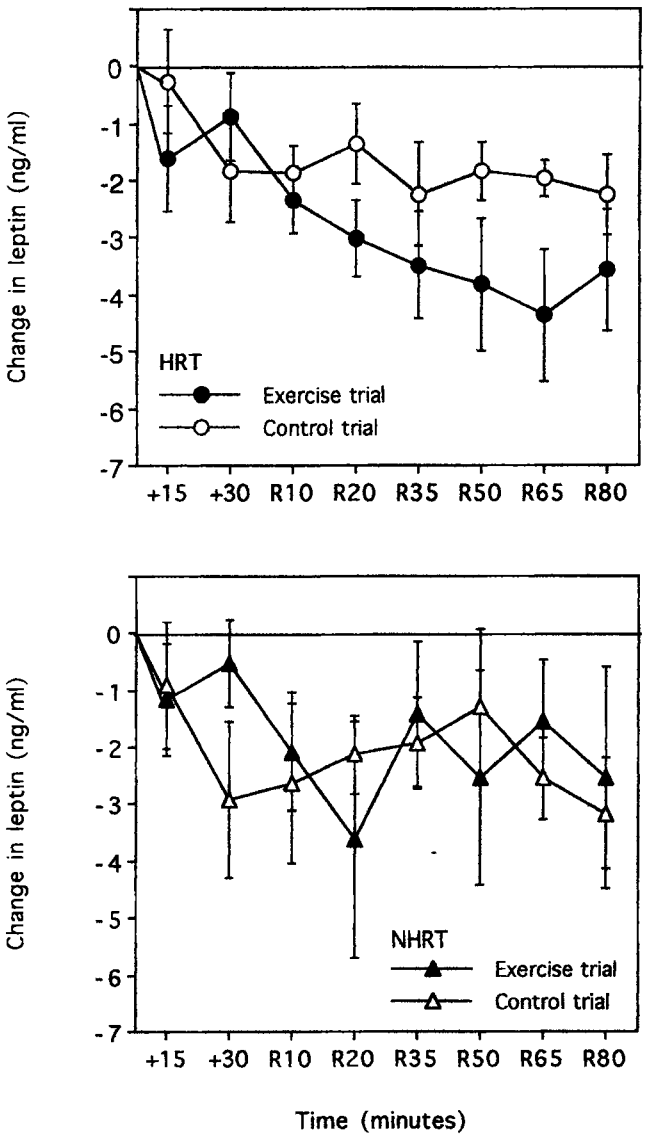

Figure 1. Data represent mean ± SEM change in leptin concentration from baseline for the HRT (n = 8) exercise (solid circles) and control (open circles) concentrations in Panel A and NHRT (n = 7) exercise (solid triangles) and control (open triangles) concentrations in Panel B.

Discussion

Results from the present study have demonstrated a reduction in serum leptin concentrations in postmenopausal women during the morning hours, regardless of HRT status or completion of a 30-min bout of moderately high intensity treadmill exercise. This is the first study to examine the

effects of HRT status on acute leptin responses to exercise in postmenopausal women. Our research design fixed the relative intensity of exercise by monitoring VO_2 and adjusting treadmill speed and grade. We also controlled for diurnal changes in leptin levels by conducting nonexercise control trials at the same time of day. Moreover, we incorporated more frequent sampling in our protocol than has been used in other response studies (e.g., 23–25) to document alterations in leptin concentrations more precisely in response to the exercise.

Whereas the decline in the leptin concentration during the exercise trial as compared to the control trial tended to be greater in the HRT group, the difference was not significant whether leptin values represented concentrations at individual time points or total leptin response (AUC). One factor that contributed to this finding was the high degree of intersubject variability. This was evident in initial resting values as well as the degree of change among subjects. A second factor that may also have affected the findings was the sample size; however, *post hoc* power analyses indicated that for relatively large effect sizes, the repeated measures design provided sufficient power to indicate statistically significantly differences.

The decline in leptin observed in our study appeared to be a result of the normal morning decline in leptin concentrations, since both exercise and control trial leptin concentrations declined. Diurnal alterations in leptin concentrations have been observed with elevated nocturnal levels followed by a decline in leptin during the morning hours (26–28). It has been observed that the circadian rhythmicity of leptin does not correlate with insulin or plasma glucose concentrations (28). Moreover, the nocturnal increases in leptin are similar to those of prolactin and thyrotropin and seem to occur prior to early morning elevation of corticotropin and cortisol (28), but whether or not neuroendocrine regulation and anterior pituitary pulsatility cause the elevation remains unknown (27). Moreover, it is unknown whether or not nocturnal increases in leptin are related to low activity levels during nighttime hours and lower leptin levels during the day are related to greater activity and energy expenditure (26).

Leptin is considered to be a signal for regulation of energy balance with higher levels suppressing NPY (1). Neuropeptide Y is known to stimulate food intake in rodents (29). Thus inhibition of NPY would produce satiety. Consequently, it could be hypothesized that exercise would result in reduced leptin concentrations, a signal that energy was expended, which would result in a reduction in satiety. The data of previous studies, mostly in men, suggest that leptin declines or does not change in response to exercise (13, 23–25). Koistinen *et al.* (13) found that exercise enhanced a "diurnal/fasting-induced fall" in leptin concentrations in men. One limitation of their study was the use of different control subjects for a nonexercise trial to account for diurnal changes. In the present study we used the same subjects for both exercise and control trials and found that a decline in leptin following exercise was due to normal diurnal changes rather than exercise. However, it is possible that if our subjects had exercised for a longer period, an exercise-induced reduction in leptin might have taken place. Our data support the idea that length of exercise is important to change in leptin concentrations.

It has been shown that leptin gene expression decreases in rats after ovariectomy and that estrogen treatment reverses this effects (10). In the same study, it was found that serum leptin levels in premenopausal women were higher than in postmenopausal women. For this reason, one could hypothesize that if leptin levels decline during exercise, higher estrogen concentrations might attenuate the decline. For another reason one could also predict that HRT would lead to the same result. It has been shown that postmenopausal women on HRT have greater blood flow to peripheral tissues during exercise than women not taking estrogen (30). Moreover, it has been suggested that there is apparently "ubiquitous expression of leptin receptors in all peripheral tissues" that may indicate metabolic regulatory function independent of hypothalamic regulation (4). It is therefore possible that greater blood flow to peripheral tissues could produce greater leptin uptake by receptors, in turn contributing to reduced circulating leptin concentrations during the exercise trial in the HRT group. Our data did not indicate any effect of estrogen replacement on serum leptin concentrations. Although leptin concentrations tended to be lower following exercise in the HRT group compared with the NHRT group, we did not find the difference to be significant.

We have reported in earlier studies that exercise caused an increase in both growth hormone (GH) and cortisol levels in the subjects of the present study (16, 17). Both of these hormones have been shown to promote leptin production (31, 32). However, insulin concentrations (not measured in the present study) decline with adequate exercise duration (33), and there is evidence that catecholamines (not measured in the present study), which rise in response to exercise (34), will inhibit leptin production (35). It is possible that a stimulatory effect of cortisol and GH was negated by decline in insulin and/or increase in catecholamines. It is also possible that a longer time period of adipocyte exposure to these hormones is required for an acute leptin response. Moreover, the extent of elevation in these hormone concentrations may not have been sufficient to produce an increase in leptin concentrations.

The present study is one of only a few that have documented responses of leptin to acute exercise. Racette *et al.* (25) found no change in serum leptin after comparing leptin levels in blood from the radial artery and the abdominal vein draining abdominal adipose tissue of five men following 1 hr of cycle exercise at 50% of predicted VO_{2max}. Exercise intensity was considerably lower than that of the present study, and the relative exercise work load was not as well quantitated as in the present study. Hickey *et al.* (23) found no change in leptin levels of 13 young male long-distance

runners following ≈ 2 hr of treadmill exercise; conversely Tuominen *et al.* (36) documented reduced leptin levels in 14 healthy young men following 2 hr of treadmill exercise that depleted muscle glycogen, but leptin levels increased after a hyperinsulinemic clamp. These studies of young males may, of course, not be applicable to the present study of postmenopausal women. Peruse *et al.* (24) found no change in plasma leptin of men and women 17–40 years of age, in response to a 10–12-min cycle ergometer-graded exercise test. Once again, the subject population was not postmenopausal, and exercise duration was considerably shorter than that of the present study. Blood samples were taken at only three periods: before, during, and immediately following the exercise test (24), which differed from the greater sampling frequency in the present study that allowed us to follow leptin concentrations over a long recovery period.

In summary, our data suggest that regardless of endocrine status, a 30-min bout of exercise does not alter serum leptin concentrations in postmenopausal women, and the observed reduction in leptin concentrations following exercise is a result of normal circadian rhythm. This response was observed regardless of HRT status. This is the first study to examine acute exercise responses to leptin concentrations in postmenopausal women and to investigate the effect of HRT in these subjects. We controlled for age, BMI, time of day, and fitness level. Future studies should focus on leptin responses to long-term exercise during which more fatty acid mobilization would occur than in the 30-min bout in the present study, but further studies should maintain the same level of control for those variables (endocrine status, time of day, etc.) as in the present study.

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