Severity of energy-related menstrual disturbances increases in proportion to indices of energy conservation in exercising women

Alterations in resting energy expenditure and metabolic hormones (energy conservation) are evident in increasing magnitude across a continuum of increasing severity of clinical menstrual disturbances, including luteal-phase defects, anovulation, and amenorrhea in exercising women. These data provide further evidence of the tight association between energy balance and reproduction and suggest that subtle declines in energy availability can produce clinically recognized menstrual disturbances. (Fertil Steril® 2007;88:971–5. ©2007 by American Society for Reproductive Medicine.)

Alterations in nutritional status and energy metabolism can have a profound modulatory effect on the reproductive axis and fertility (1). In humans, the suppression of reproductive function with chronic energy deficiency is more strongly predicted by the magnitude of a daily energy deficit rather than by loss in body mass (2). These findings are consistent with the hypothesis that acute fuel availability, not chronic energy stores, is a key modulator of reproductive function (1). Sustained reductions in resting energy expenditure (REE), presumably in an attempt to conserve energy, have been observed in exercising women with amenorrhea compared with their normally cycling counterparts (3). These changes have been observed in concert with other endocrine adaptations that reflect an energetically challenged condition, including decreased concentrations of triiodothyronine (TT3), leptin, insulin, insulin-like growth factor-1 (4–7), and increased counterregulatory hormones such as growth hormone, ghrelin, and cortisol (4, 8, 9).

Although energy-related menstrual disturbances in athletes and physically active women have been described as existing along a continuum, ranging from subtle perturbations like luteal phase defects (LPDs) and anovulatory cycles, to the more severe perturbations, oligomenorrhea and amenorrhea (10), the majority of metabolic and energy studies has been limited to amenorrheic athletes. To this end, the purpose of this study was to determine whether menstrual disturbances along a continuum of severity are associated with a concomitant range of severity in indices of energy conservation, defined as decreased REE per kilogram of fat-free mass (FFM) and alterations in metabolic hormones consistent with adaptation to energy deficiency. We hypothesized that because menstrual disturbances exist on a continuum, indices of energy conservation would be observable even when changes in menstrual cyclicity are subtle and that these indications of energy conservation should increase in magnitude as the severity of the menstrual disturbance increases. If our hypothesis is correct, this finding would underscore the importance of adequate energy for fertility and the maintenance of endocrine homeostasis in exercising women.

Forty-nine volunteers participated in the study and were retrospectively grouped according to exercise status and by menstrual status. Menstruating women were monitored for two to three menstrual cycles, and amenorrheic women, for two to three 30-day monitoring periods. All data presented in this study represent the mean of the two to three repeated time periods monitored. The study was approved by the Ethics Review Board at the University of Toronto, and all volunteers signed an approved informed consent document.

Exercise status was defined as sedentary when purposeful exercise was <2 hours per week and as exercising when purposeful exercise was >2 hours per week (11, 12). Menstrual status was determined from daily first-morning void urine samples assayed for LH, pregnanediol 3-glucuronide, and estrone 3-glucuronide (13). Menstrual categories from these daily urinary measures included ovulatory, LPD, anovulatory, and amenorrheic. After consideration of exercise status (sedentary or exercising) and menstrual status, volunteers were grouped as follows: [1] sedentary ovulatory group (SedOv, n = 11) with consistently ovulatory cycles for the duration of the 2- to 3-month study period; [2] exercising ovulatory group (ExOv, n = 9) with consistently ovulatory cycles for the duration of the study period; [3] exercising with inconsistent presentations of subtle menstrual disturbances group (ExIncon, n = 8), including various
Menstrual cycle length, follicular and luteal phase length, ovulatory status, and the presences or absence of LPD was defined as reported elsewhere (12). Amenorrhea was defined as no menses for ≥100 days, confirmed by estrone 3-glucuronide and pregnanediol 3-glucuronide profiles.

Total body mass, height, body mass index, and body composition (Prodigy, GE Lunar, enCORE 2002 software, version 6.50.069) were measured. Peak oxygen uptake (VO₂ peak) was measured once, as defined elsewhere (14).

Eight-hour–fasted blood samples were collected between 7:30 AM and 10:00 AM for hormone assays two to three times by indirect calorimetry by using methods reported elsewhere using the Weir equation (14, 15), and the average is reported (corrected for FFM).

Microtitre plate competitive enzyme immunoassays were used to measure estrone 3-glucuronide and pregnanediol 3-glucuronide by using polyclonal antibodies supplied by another investigator (16, 17). Urine samples were corrected for specific gravity (18). Urinary LH was determined by RIA (Diagnostics Products Corporation, Los Angeles, CA). Eight-hour–fasted blood samples were collected between 7:30 AM and 10:00 AM for hormone assays two to three times during the study period, and we report the average of these repeated measurements. Triiodothyronine (DPC), and leptin (Linco Research, St. Charles, MO) were measured by using an immunoassay, and total ghrelin was measured by using an RIA (Linco Research).

All data sets were tested for nonnormality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. Outliers detected were rejected. Data were expressed as mean ± SEM. Data for all variables were compared among the groups by using analysis of variance. When main effects were found, post hoc tests (least significant difference) were used to detect where differences occurred. Forward stepwise regression was used to determine predictors of REE. The criteria for entry into and removal from the model were \( P < .05 \) and \( P < .10 \), respectively. The number of volunteers included in this study was sufficient to detect significant differences caused by our grouping variable (exercise and menstrual status) at a power level of 0.80 by using a \( P \) level of .05. Significance level of <.05 was chosen to identify all significant differences and was adjusted for multiple comparisons. All data were analyzed by using SPSS for Windows (version 12.0; Chicago, IL).

Age (24.8 ± 1.4 y) height (165.1 ± 1.5 cm), body mass (58.0 ± 1.9 kg), body mass index (21.3 ± 0.8 kg/m²), and gynecological age (12.1 ± 1.1 y) were not significantly different among the groups. All exercising groups had a lower percentage of body fat (\( F = 4.40, P = .005 \)) than did the SedOv group (22.5 ± 1.9 vs. 30.5 ± 2.1%). Total exercise volume (336.0 ± 59.3 vs. 101.9 ± 23.8 min/wk, \( F = 4.091, P = .008 \)) and VO₂ peak (45.4 ± 1.8 vs. 39.2 ± 1.3 mL/kg per minute, \( F = 4.918, P = .003 \)) were higher in all exercising groups compared with the SedOv group.

Composite graphs of the study group’s cycles are presented in Figure 1. Average menstrual cycle length (28.9 ± 1.1 d), follicular phase length (17.1 ± 1.7 d), and luteal phase length (12.2 ± 0.6 d) were not significantly different among the menstruating sedentary and exercising groups. The average duration of amenorrhea before the study in the ExAmen subjects was 215.8 ± 40.1 days.

Resting energy expenditure and metabolic hormone data are presented in Figure 1. All groups with menstrual disturbances, including the ExAmen, ExAnov, and ExIncon groups, had a significantly lower REE/FFM compared with the SedOv group, and the ExAmen had a significantly lower REE/FFM than the ExOv group. Stepwise forward regression was used to predict REE/FFM. Variables in the model predicted 45.3% (\( R^2 = 0.453 \)) of the variance in REE/FFM (\( F = 17.4; P < .0001 \)). Menstrual status category was the most powerful predictor, accounting for 32.5% of the variance (\( R^2 = 0.325 \)), whereas leptin accounted for an additional 12.8% of the variance at step 2 (\( R^2 \) change = 0.128).

Serum TT₃ levels were lower (\( F = 4.830, P = .003 \)) in all groups with menstrual disturbances, including the ExAmen, ExAnov, and ExIncon groups, compared with the SedOv group. Leptin levels were lower (\( F = 3.57, P = .014 \)) in all exercising groups compared with the SedOv group. There was a trend toward higher ghrelin levels in the ExAmen group compared with the other groups (\( F = 2.51, P = .092 \)). The REE/FFM was correlated with TT₃ (\( r = 0.474, P < .001 \)), ghrelin (\( r = -0.410, P = .002 \)), and leptin (\( r = 0.533, P < .001 \)).

This study examined indices of energy status, including REE and metabolic hormones, TT₃, ghrelin, and leptin, across the continuum of energy-related menstrual disturbances in physically active women, and it included both recreational athletes and competitive athletes. We found that alterations in REE and metabolic hormones were consistent with adaptations to a chronic energy deficiency and that these alterations were distributed across the continuum of clinical menstrual disturbances in accordance with the magnitude of severity of the menstrual dysfunction observed. This study illustrates the sensitivity of this association and provides support for the existence of a dose–response relationship between laboratory measures of energy status, i.e., REE, TT₃, leptin and ghrelin, and clinical categories of menstrual dysfunction, i.e., LPD, anovulation, and amenorrhea. These data suggest that even subtle changes in energy availability may be translated to the reproductive axis, i.e., that energy deficiency is associated with delays in follicular
(A) Bar graph of REE per kilogram of FFM (kcal/d of REE per kg of FFM), TT3 (nmol/L), ghrelin (pg/mL), and leptin (µg/L) in the sedentary and exercising women grouped by menstrual status. Values are mean ± SEM. Significant differences are denoted as follows: *ExAmen, ExAnov, ExIncon vs. SedOv; †ExAmen vs. ExOv; ‡ExAmen vs. SedOv, ExOv, ExIncon, ExAnov; and §ExAmen, ExAnov, ExIncon, ExOv vs. SedOv. (B) Composite graph of menstrual status depicted by daily estrone 3-glucuronide (E1G) and pregnanediol 3-glucuronide (PdG) concentrations in the sedentary and exercising women grouped by menstrual status. The E1G and PdG data for SedOv, ExOv, and ExIncon groups are aligned by the day of the LH peak, defined as day 0. The anovulatory (ExAnov) and amenorrheic (ExAmen) subjects’ E1G and PdG data are aligned by chronological day of daily urinary hormone collections. The number of days depicted for the amenorrheic subjects is the mean cycle length of the menstruating subjects. Values are mean ± SEM. SedOv = sedentary ovulatory; ExOv = exercising ovulatory; ExIncon = exercising ovulatory, luteal phase defect and anovulatory inconsistent cycles; ExAnov = exercising anovulatory; ExAmen = exercising amenorrheic.

Whereas TT₃ and REE/FFM decreased in magnitude with energy deficit and increasingly severe estrogen deficiency (10). This point is important because clinical sequelae such as infertility and bone loss become exacerbated in the face of an energy deficit and increasingly severe estrogen deficiency. A sustained reduction in serum TT₃ and REE/FFM was observed in our data, not only in the exercising women with amenorrhea as observed elsewhere (20), but also in the exercising women with mild to moderate menstrual disturbances, i.e., LPD and anovulatory menstrual cycles. In our work published elsewhere (20) in a different group of exercising women with LPD, we also reported lower concentrations of TT₃. One other study has reported a reduced REE/FFM in exercising women with either LPD or anovulatory cycles (21), but menstrual disturbances were categorized from salivary samples of P that were collected every other day for only one menstrual cycle. In this study, we have very precisely characterized menstrual function by the quantification of daily urinary excretion of the ovarian steroids estrone 3-glucuronide and pregnanediol 3-glucuronide for at least two menstrual cycles, and we have carefully discriminated ovulatory cycles from LPD and anovulatory cycles. This point is important because clinical sequelae such as infertility and bone loss become exacerbated in the face of a chronic energy deficiency and menstrual cycle disturbances in exercising women. The primary findings of this study demonstrate that [1] alterations in REE and metabolic hormones were distributed across the continuum of clinical menstrual disturbances in accordance with the severity of the menstrual dysfunction observed, providing evidence that energy-related menstrual disturbances increase in severity proportional to the magnitude of increase in energy conservation; [2] a reduction in serum TT₃ concentration likely promotes the energy conservation through the lowering of REE, an effect that is coupled with a paradoxical elevation in total ghrelin; [3] this relationship was not exclusive to the most severe menstrual disturbance, amenorrhea, and the fact that it also was observed in the exercising women with consistent subtle and less severe menstrual dysfunction such as anovulation provides key evidence that a progressive metabolic adjustment exists before the onset of amenorrhea; and [4] this study illustrates the sensitivity of the association between metabolic status and reproductive function and provides support for the existence of a dose–response relationship between laboratory measures of energy status, such as REE and metabolic hormones, and clinical categories of menstrual dysfunction, that is, LPD and anovulation, suggesting that even subtle changes in energy availability may be translated to the reproductive axis and associated with delays in follicular maturation and compromised luteal function.

The observed shifts in metabolic hormones and REE observed in this study are not exclusive to high-performance athletes; women engaged in physical activity for recreational (or occupational) purposes are impacted also. Independent of the level of activity that exercising women may choose, it is ultimately the combination of inadequate nutritional intake relative to energy expenditure that initiates this metabolic cascade.

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