

Reduced Catecholamine Response to Exercise in Amenorrheic Athletes

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ABSTRACT

SCHAAL, K., M. D. VAN LOAN, and G. A. CASAZZA. Reduced Catecholamine Response to Exercise in Amenorrheic Athletes. *Med. Sci. Sports Exerc.*, Vol. 43, No. 1, pp. 34–43, 2011. Studies have found an array of endocrine disturbances related to energy deprivation in women with functional hypothalamic amenorrhea. **Purpose:** We examined the catecholamine response to exercise in five eumenorrheic (EU) and five amenorrheic (AM) athletes, matched by age (mean \pm SEM: EU = 29.8 \pm 2.5 yr and AM = 31.0 \pm 4.3 yr) and running volume (EU = 56.4 \pm 8.1 km \cdot wk⁻¹ and AM = 61.5 \pm 6.4 km \cdot wk⁻¹). **Methods:** Subjects performed a maximal treadmill test followed by a 30-min recovery and then a submaximal running test, consisting of 4-min stages at 60%, 70%, and 80% and 15 min at 85% of peak oxygen consumption ($\dot{V}O_{2peak}$). Blood was drawn after each stage to measure glucose, lactate, epinephrine, norepinephrine, and cortisol concentrations. HR, blood pressure, and rate of perceived exertion were also measured at each stage. **Results:** There were no differences between groups in body composition or $\dot{V}O_{2peak}$ (EU = 57.3 \pm 2.3 mL \cdot kg⁻¹ \cdot min⁻¹ and AM = 54.1 \pm 1.2 mL \cdot kg⁻¹ \cdot min⁻¹). Resting HR and mean arterial pressure were significantly ($P \leq 0.05$) lower in AM. Norepinephrine was lower in AM at 70%, 80%, 85%, and 100% of $\dot{V}O_{2peak}$ (EU = 7784.5 \pm 582.9 pg \cdot mL⁻¹ and AM = 3626.1 \pm 271.4 pg \cdot mL⁻¹ at $\dot{V}O_{2peak}$). Epinephrine (EU = 1470.3 \pm 275.1 pg \cdot mL⁻¹ and AM = 416.9 \pm 67.5 pg \cdot mL⁻¹) and blood lactate (EU = 10.1 \pm 1.2 mmol \cdot L⁻¹ and AM = 6.7 \pm 0.9 mmol \cdot L⁻¹) were lower at $\dot{V}O_{2peak}$ in AM. **Conclusions:** Our results demonstrate a reduced adrenergic response to intense exercise in AM athletes as indicated by reduced blood lactate and catecholamine concentrations. A suppressed catecholamine response could decrease performance by reducing the sympathetic drive essential for the cardiovascular and metabolic adjustments needed to maintain high intensities of exercise. **Key Words:** CORTISOL, EPINEPHRINE, NOREPINEPHRINE, LACTATE, PEAK OXYGEN CONSUMPTION

Over the last decade, much attention has been paid to the prevalence of functional hypothalamic amenorrhea (FHA) in female athletes, seen most often within competitive sports that emphasize leanness as a factor to successful performance (14,31). Many studies have supported low energy availability (EA) as the cause of FHA, whether this energy deficiency is due to high rates of energy expenditure, insufficient food intake, or both (22,27,29,49). As one of the most expensive nonessential metabolic processes, reproductive function is sensitive to overall energy status (47). Hypothalamic activity is suppressed in response to caloric deprivation, resulting in disturbed patterns of gonadotropin-releasing hormone, luteinizing hormone, and estrogen production (30–32,47). The entire hypothalamic–pituitary–gonadal axis is therefore disrupted.

Much of the research on the physiology of amenorrheic (AM) athletes has focused on endocrine-metabolic aberra-

tions other than those directly related to reproductive dysfunction, with most disturbances occurring in relation to the hypothalamic–pituitary axis (24). Women with FHA have displayed mild hypercortisolism, hypothyroidism, hypoinsulinemia, and mild hypoglycemia (24,29,41). In addition, studies have found a decreased resting metabolic rate in AM women (25). Together, these changes point to a catabolic, energy-conserving physiological state in AM women, supporting the notion that negative energy balance results in profound endocrine and metabolic changes to preserve fuel stores. Knowledge of the consequences of caloric deficiency in AM athletes raises concern about its potential impact on exercise metabolism and athletic performance. Indeed, as intense and prolonged exercise presents a challenge to glucose homeostasis, an appropriate sympathetic and glucocorticoid response is important to maintain euglycemia (24).

In extreme states of fatigue or glycogen depletion, the counterregulatory response to exercise may be impaired, forcing the individual to quit exercising or to reduce workload to protect vital fuel stores. This has been documented in glycogen-depleted and chronically overtrained athletes, who exhibited suppressed neuroendocrine responses to intense exercise and a concomitant drop in performance (44). In response to insulin or exercise-induced hypoglycemia, cortisol released by the adrenal cortex binds to corticosteroid receptors in the brain (18), triggering a suppression of the autonomic counterregulatory response. This mechanism,

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known as the hypoglycemia-associated autonomic failure (HAAF), has been reported to occur in healthy men but not in healthy premenopausal women (5,6,16). Galassetti et al. (15,16) found that after antecedent hypercortisolism, the HAAF-induced suppression of the catecholamine response to exercise was more important in men than that in women. The ability of estrogen to bind to these brain corticosteroid receptors in rats (12,43) may explain this gender difference, thereby reducing the magnitude of the HAAF phenomenon in women with normal estrogen levels. To our knowledge, no one has examined whether AM women, subjected to consistent hypoestrogenism, experience HAAF. It is worth considering that the combination of low EA and hypoestrogenism may subject women with FHA to HAAF and result in a blunted autonomic response to exercise.

Although studies have documented a mild hypercortisolism at rest and a suppressed cortisol response to exercise in AM athletes (8,9), whether the catecholamine response is reduced during exercise remains uncertain. The only study to report catecholamine levels with exercise did not find a difference in epinephrine or norepinephrine levels between AM and eumenorrheic (EU) athletes (28). However, the blood samples analyzed had been drawn 4 min after exercise at 85% $\dot{V}O_{2\max}$, well beyond the 1- to 2-min half-life for catecholamines. Therefore, the aim of this study was to determine whether the sympathetic response during maximal and high submaximal exercise differed between AM and EU runners. We hypothesized that AM athletes would exhibit lower EA and a reduced sympathetic response to intense exercise compared with their EU counterparts.

METHODS

Subjects. Twelve competitive, endurance-trained EU (6) and AM (6) female athletes were recruited for the study from the university and surrounding communities. Although there are data comparing men and women (50) and different phases of the menstrual cycle (1) in EU women and after exercise in AM athletes (28), data on catecholamine responses to exercise in athletes with contrasting reproductive function are rare. Therefore, we sized our study to detect catecholamine levels in AM athletes that were two times below the normal range in EU athletes. The number of subjects required per sample in a two-sample test was as follows: $N = 2(t_{\alpha}, df + t_{\beta}, df)2 (s/d)2$ or $N = 2(t_{0.05, 8 + t_{0.20, 8}2 (1/2)2$ or $N = 2(2.31 + 0.89)2 (1/2)2 = 5.1$, which rounds down to five subjects per group. Therefore, we determined that 12 subjects were adequate for detecting differences in catecholamines because of menstrual cycle differences. However, only five subjects from each group were included in the data analysis. One AM athlete sustained an injury that did not allow her to complete all of the exercise testing, and one EU subject divulged after testing that she had endoscopic thoracic sympathectomy before the study. Healthy, premenopausal women were matched by age, self-reported running miles per week,

and main endurance event, ranging from 5-km races to Ironman™ triathlons. Athletes who ran 32 km or more per week were recruited for the study. Subjects were non-smokers, had not been pregnant or lactating within the past 18 months, and had not taken oral contraceptives for at least 6 months. All women were in overall good health as assessed by a health and training history questionnaire and an examination by a licensed physician. Subjects completed an informed consent approved by the institutional review board of the University of California at Davis (200715299-1).

Menstrual status. Information on menstrual history and age at menarche were collected by health history questionnaire. Subjects were placed into groups on the basis of menstrual history with AM classified as 3 or less menstrual cycles in the last year and EU as 10 or more menstrual cycles in the last year. AM subjects provided documentation from their physician that they had been diagnosed with functional amenorrhea that was not likely caused by an endocrine or a physical abnormality, and all subjects had normal pelvic exams. Subjects with primary amenorrhea were excluded. To monitor eating behavior, the Eating Disorder Evaluation Questionnaire (EDE-Q) (10) was administered. The EDE-Q is a self-report questionnaire composed of four subscales (weight concern, shape concern, eating concern, and dietary restraint), and the global score is the mean score of the four subscales.

Experimental protocol. Subjects reported to the laboratory on two occasions, between 7:30 and 8:30 a.m., after a day of rest or light training. They were asked to be well hydrated and to eat their usual prerace meals over the 30 h (breakfast, lunch, dinner for the day prior, and breakfast for the day of testing) preceding each test session to ensure that they were not hypoglycemic and had adequate glycogen stores before exercise. Thirty-hour pretest dietary intake was recorded and analyzed for energy intake (EI) and macronutrients using Food Processor SQL (Version 9.2.0; ESHA Research, Salem, OR). Subjects only consumed water (200–300 mL) during the testing session. The first laboratory visit was a practice session, and no blood samples were taken. Medical clearance was obtained at this time. The second session for the EU subjects was scheduled during the follicular phase (days 1–8 after the start of menses) when estrogen and progesterone levels are low and more closely resemble the low values of the AM women.

Resting measurements. Subjects arrived at the laboratory 1–2 h after consuming their usual prerace breakfast. Subjects' height and weight were measured, and body composition was determined via dual-energy x-ray absorptiometry (DEXA, Lunar Prodigy; GE Healthcare, Madison, WI). Before exercise testing, subjects were asked to complete the POMS questionnaire (34) as a measure of overall mood disturbance. The POMS questionnaire consisted of 65 questions asking subjects to describe feelings they were having with regard to tension, depression, anger, vigor, fatigue, and confusion. Scores were obtained for each of the six categories, and then all except the vigor score were

combined and subtracted from the vigor score to get a total mood disturbance score. A 22-G catheter was then inserted into a wrist or a forearm vein while the subjects rested quietly on an examination table. Subjects rested for 10 min after catheterization to allow for normalization of catecholamine levels because of the catheter insertion. Because cortisol secretion follows diurnal fluctuations, we obtained all resting cortisol samples between 8 and 9 a.m.

Peak exercise test. All exercise tests were performed on the same treadmill set at a 1% grade (Stairmaster Clubtrack 2100 LE; Nautilus, Vancouver, WA). After an 8- to 12-min warm-up, subjects performed a continuous graded exercise test to exhaustion to measure peak oxygen consumption ($\dot{V}O_{2\text{peak}}$). The initial running speed was chosen on the basis of recent race performance and expected maximal speed to optimize the duration of the test at 12–15 min. Speed increased by $1.56 \text{ km}\cdot\text{h}^{-1}$ every 2 min for the first 6 min then by $0.8 \text{ km}\cdot\text{h}^{-1}$ every 2 min until exhaustion. A metabolic cart (TrueOne 2400; ParvoMedics, Sandy, UT) was used to monitor gas exchange. HR was monitored throughout the test using an HR monitor (5410; Polar, Woodbury, NY), and RPE was recorded at the end of each stage using a 10-point scale (36). Immediately after the maximal treadmill test, peak blood pressure (BP) was measured manually with a single-hosed sphygmomanometer and stethoscope, while a blood sample was obtained for peak lactate, glucose, and hormone concentrations. An active 6 min cool down and 30 min rest period ensued.

Submaximal exercise test. To measure hormonal and metabolic responses to various running intensities, subjects performed a noncontinuous graded exercise test after a 30-min recovery period after the maximal treadmill test. The maximal exercise test was performed first to obtain a true maximal effort. Gas exchange, HR, and RPE were again monitored. From the $\dot{V}O_{2\text{peak}}$ results, running speeds corresponding to 60%, 70%, 80%, and 85% of $\dot{V}O_{2\text{peak}}$ were calculated. For the first three stages, subjects ran for 4 min or until steady state, and for the last stage, at 85% of $\dot{V}O_{2\text{peak}}$, subjects ran 15 min to measure the hormonal and metabolic responses to a slightly prolonged high submaximal effort above their ventilatory threshold. At the end of each stage, subjects stopped briefly (about 1 min) while a blood sample was collected and BP was measured.

Blood analysis. Blood samples were collected in non-heparinized syringes. One drop of blood was used to measure blood glucose concentrations with a portable glucose analyzer (Accu-Check; Roche, Mannheim, Germany) and another drop of blood to measure lactate concentration using a portable lactate analyzer (Lactate Pro; Arkray, Inc., Kyoto, Japan), microhematocrit tubes (Statspin, Norwood, MA) were used to determine hematocrit, 3 mL of blood was placed into ethylenediaminetetraacetic acid tubes for epinephrine and norepinephrine analysis, and 2 mL of blood was placed into serum separator tubes (SST) tubes for cortisol analysis. The tubes were immediately placed on ice and centrifuged within 20 min of collection. The plasma and the

serum samples were aliquoted and stored at -80°C until analysis.

Epinephrine and norepinephrine concentrations were determined via enzyme immunoassay (Alpco Diagnostics, Salem, NH). Samples, standards, and controls were analyzed in duplicates. Intra-assay coefficients of variation = 9.8% and 6.9% for norepinephrine and epinephrine, respectively. The absorbance was read using a microplate reader (Synergy™ 2; Biotek Instruments, Inc., Winooski, VT) set to 450 nm. Calibration curves were obtained from the standards using spline, a nonlinear regression for curve fitting. Cortisol concentrations were determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite® 1000; Siemens Healthcare Diagnostics Inc., Deerfield, IL). Samples and standards were analyzed in duplicate (intra-assay coefficients of variation = 6.7% and 8.8%).

Energy availability. Over the course of 7 d, subjects recorded all food and fluid consumption as well as exercise training sessions, including duration, HR, modes, and intensity using the 10-point RPE scale. Exercise energy expenditure (ExEE) in kilocalories per minute was estimated on the basis of RPE and HR during training to matched oxygen consumption and RER during laboratory testing. Energy intake (EI) was analyzed from the 7-d food records for kilocalories and macronutrients using Food Processor SQL (Version 9.2.0; ESHA Research). Energy availability (EA) was calculated as $\text{EI} - \text{ExEE}$ corrected for lean body mass. Subjects also wore armband activity monitors, a multidirectional accelerometer (Sensewear Pro 3; Body Media Inc., Pittsburgh, PA) that recorded average energy expenditure in calories expended per day.

Statistical analysis. Data are presented as mean \pm SEM for EU and AM groups. Unpaired *t*-tests were performed to assess group differences in anthropometric, training, nutrition, and activity characteristics as well as cardiovascular, metabolic, and hormonal traits at rest and maximal physical effort (StatView software Version 5.0.1; SAS Institute Inc., Cary, NC). Two-way repeated-measures ANOVA and Fisher's protected least significant difference (PLSD) *post hoc* test were used to determine differences in the cardiovascular, endocrine, and metabolic variables over time in response to exercise. Simple regressions were used to determine correlations, and stepwise regression analysis was used to determine which independent variables were significant contributors to the catecholamine and blood lactate response to peak exercise. Significance was set at $P \leq 0.05$.

RESULTS

Subject characteristics. Anthropometric and training characteristics of the subjects are shown in Table 1. There were no significant differences in age, height, weight, body composition, or age at menarche. Years of regular endurance training and self-reported weekly running miles did not differ between groups. There were no group differences

TABLE 1. Subject characteristics.

Variable	EU	AM	P Value
Age (yr)	30 ± 2.5	31 ± 4.3	0.81
Anthropometric characteristics			
Height (cm)	170 ± 2.5	170 ± 1.7	0.78
Weight (kg)	59 ± 3.6	55 ± 2.5	0.36
Body fat (%)	17 ± 1.9	14 ± 2.5	0.25
LBM (kg)	48 ± 2.2	47 ± 2.1	0.69
Fat mass (kg)	10 ± 1.7	7.4 ± 1.5	0.22
Menstrual characteristics			
Age of menarche (yr)	14 ± 0.9	13 ± 0.4	0.51
Menstrual cycles per year	12 ± 0.9	0.6 ± 0.6	0.0001*
Length of menstrual cycle (d)	30 ± 2.5		
Years with amenorrhea		2.6 ± 0.3	
Training characteristics			
Years of training	7.5 ± 3.2	9.6 ± 3.3	0.66
Running distance (km·wk ⁻¹)	56 ± 8.8	62 ± 7.0	0.66
VO _{2peak}			
L·min ⁻¹	3.4 ± 0.2	3.1 ± 0.2	0.25
mL·kg ⁻¹ ·min ⁻¹	57 ± 2.3	56 ± 1.5	0.61
mL·kg ⁻¹ LBM·min ⁻¹	69 ± 2.1	65 ± 2.8	0.23
Speed at peak (km·h ⁻¹)	17 ± 0.5	16 ± 0.6	0.43

Values are presented as mean ± SEM for five EU and five AM women.

* Significantly different from EU; $P < 0.05$.

VO_{2peak}, peak rate of oxygen consumption; LBM, lean body mass.

in peak oxygen consumption either in absolute liters per minute or relative to body weight or lean body mass. EDE-Q scores are listed in Table 2. Although AM scored within 1 SD of the young women's norms for the EDE-Q in all categories (10), they had significantly higher global scores and shape concern scores than EU. There were no significant differences ($P > 0.1$) in any of the categories of the POMS questionnaire between groups, and both groups displayed a healthy "iceberg profile" typical of athletes (35) (Fig. 1): total mood disturbance = -8.0 ± 6.1 in EU versus 6.0 ± 7.1 in AM, $P = 0.09$; tension = 4.2 ± 1.2 in EU versus 8.0 ± 1.1 in AM, $P = 0.18$; depression = 0.8 ± 0.8 in EU versus 1.2 ± 1.2 in AM, $P = 0.59$; anger = 1.0 ± 0.8 in EU versus 1.8 ± 0.7 in AM, $P = 0.66$; vigor = 19.4 ± 3.2 in EU versus 15.4 ± 1.6 in AM, $P = 0.37$; fatigue = 1.8 ± 0.5 in EU versus 5.4 ± 2.3 in AM, $P = 0.27$; and confusion = 1.8 ± 0.4 in EU versus 4.6 ± 2.0 in AM, $P = 0.15$.

EI and energy expenditure. The 30-h pretest diet recall analysis (breakfast, lunch, dinner for the day prior, and breakfast for the day of testing) indicated no differences ($P > 0.2$) in caloric intake (2800 ± 300 vs 2600 ± 200 kcal), carbohydrate ($57\% \pm 4\%$ vs $53\% \pm 4\%$), protein ($12\% \pm 1\%$

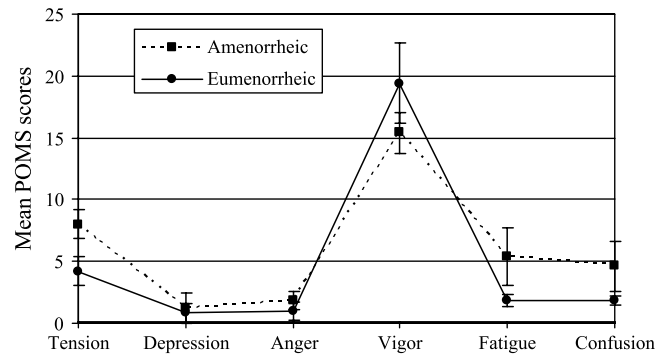


FIGURE 1—Overall mood profiles from the POMS questionnaires.

vs $16\% \pm 1\%$), or fat ($30\% \pm 4\%$ vs $28\% \pm 3\%$) consumption between AM and EU. EI over the 7-d recording period in absolute and per kilogram of lean body mass was similar between groups as was the macronutrient composition (Table 3). We were unable to measure energy expenditure during routine activities at the same time on nonexercise days and thus most likely have ExEE that are about $2 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM} \cdot \text{d}^{-1} \cdot \text{h}^{-1}$ of exercise higher than those reported by Loucks and Thuma (31). However, for practical purposes, this difference is negligible. There were no significant differences in total energy expenditure, ExEE, or EA between groups over the 7-d recording period (Table 3). However, the EA in AM subjects tended to be lower than $30 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM} \cdot \text{d}^{-1}$, according to a one-sample t -test with the hypothesized mean set at 30 instead of 0 ($P = 0.07$). Further, ExEE per lean body mass tended to be higher in AM ($P = 0.08$), and the percentage of energy expenditure from exercise out of total energy expenditure was 18% higher in AM ($P = 0.04$).

Resting cardiovascular, metabolic, and hormonal parameters. Cardiovascular, metabolic, and endocrine values at rest are presented in Table 4. Resting HR and mean arterial pressure (MAP) were significantly lower in AM than EU. Resting blood glucose, lactate, hematocrit, and hemoglobin levels were not different between groups. Resting plasma norepinephrine, epinephrine, and cortisol concentrations also did not differ between groups.

TABLE 3. Average daily EI, energy expenditure, exercise, and EA.

	EU	AM	P Value
EI (kcal·d ⁻¹)	2200 ± 82	2100 ± 247	0.90
EI (kcal·kg ⁻¹ LBM·d ⁻¹)	45 ± 2.8	46 ± 5.1	0.97
% EI as carbohydrate	52 ± 2.5	57 ± 3.3	0.25
% EI as fat	28 ± 2.4	28 ± 2.6	0.86
% EI as protein	15 ± 1.0	13 ± 1.2	0.23
% EI as alcohol	4.4 ± 1.1	2.1 ± 1.3	0.21
Running volume (h·wk ⁻¹)	4.5 ± 1.3	6.0 ± 0.7	0.34
Overall training volume (h·wk ⁻¹)	9.3 ± 1.8	14 ± 3.0	0.21
EE (kcal·d ⁻¹)	2600 ± 190	2700 ± 230	0.77
EE (kcal·kg ⁻¹ LBM·d ⁻¹)	54 ± 3.3	57 ± 3.3	0.52
Exercise EE (kcal·d ⁻¹)	800 ± 132	1300 ± 293	0.12
Exercise EE (kcal·kg ⁻¹ LBM·d ⁻¹)	16 ± 2.8	28 ± 5.1	0.08
% total EE as exercise	29 ± 3.8	48 ± 6.3	*0.04
EA (kcal·kg ⁻¹ LBM·d ⁻¹)	29 ± 4.8	18 ± 6.6	0.21

Values are presented as mean ± SEM for five EU and five AM women.

* Significantly different from EU; $P \leq 0.05$.

EE, energy expenditure; LBM, lean body mass.

TABLE 2. EDE-Q questionnaire scores.

Variable	EU	AM	P Value	Norm Values
EDE-Q				
Global score (scale from 0 to 6)	0.2 ± 0.1	1.3 ± 0.3*	0.01	1.6 ± 1.2
Dietary restraint score (scale from 0 to 6)	0.3 ± 0.1	1.5 ± 0.5	0.08	1.3 ± 1.3
Eating concern score (scale from 0 to 6)	0.1 ± 0.5	1.1 ± 0.5	0.09	0.6 ± 0.9
Shape concern score (scale from 0 to 6)	0.3 ± 0.1	1.6 ± 0.3	0.01*	2.1 ± 1.6
Weight concern score (scale from 0 to 6)	0.3 ± 0.2	0.8 ± 0.2	0.13	1.6 ± 1.4

Values are presented as mean ± SEM for four EU and five AM women.

Norm values are presented as mean ± SD (10).

* Significantly different from EU; $P \leq 0.05$.

TABLE 4. Resting cardiovascular and blood parameters.

Variable	EU	AM	P Value
HR (bpm)	55 ± 0.6	44 ± 3.5*	0.01
Systolic BP (mm Hg)	110 ± 1.3	110 ± 4.4	0.26
Diastolic BP (mm Hg)	68 ± 2.5	62 ± 2.1	0.09
MAP (mm Hg)	83 ± 1.7	77 ± 1.9*	0.05
Glucose (mmol·L ⁻¹)	5.1 ± 0.5	4.1 ± 0.3	0.10
Lactate (mmol·L ⁻¹)	1.5 ± 0.1	1.5 ± 0.2	0.72
Hematocrit (%)	39 ± 1.1	39 ± 0.9	0.84
Hemoglobin (gm·dL ⁻¹)	13 ± 0.3	14 ± 0.4	0.75
Epinephrine (pg·mL ⁻¹)	40 ± 7.0	76 ± 22	0.15
Norepinephrine (pg·mL ⁻¹)	357 ± 161	430 ± 131	0.73
Cortisol (μg·dL ⁻¹)	13 ± 2.2	15 ± 1.5	0.20

Values are presented as mean ± SEM for five EU and five AM women.

* Significantly different from EU; $P \leq 0.05$.

Exercise cardiovascular, metabolic, and hormonal parameters. Exercise cardiovascular and blood parameters are presented in Table 5. There were no group differences in speed, oxygen consumption, RER, RPE, HR, MAP, blood glucose, or cortisol. At each submaximal stage, $\dot{V}O_2$ of all AM and EU subjects matched the desired 60%, 70%, 80%, and 85% of $\dot{V}O_{2peak}$. RER was identical in both groups, and all subjects indicated maximal exertion with an RPE of 10. HR and systolic BP increased with rising workloads, whereas diastolic BP did not change. As a result, MAP only rose slightly from its resting value during exercise. Resting glucose levels were maintained through the 80% workload, increasing in both groups only after 15 min at 85% of $\dot{V}O_{2peak}$. Hematocrit did not differ ($P > 0.2$) between groups during exercise ($42.0\% \pm 1.2\%$ vs $42.6\% \pm 0.8\%$ at 60% $\dot{V}O_{2peak}$, $43.4\% \pm 1.5\%$ vs $41.6\% \pm 1.2\%$ at 70% $\dot{V}O_{2peak}$, $42.4\% \pm 1.6\%$ vs $41.2\% \pm 1.8\%$ at 80% $\dot{V}O_{2peak}$, $46.0\% \pm 2.4\%$ vs $43.4\% \pm 0.7\%$ at 85% $\dot{V}O_{2peak}$, and $45.4\% \pm 2.2\%$ vs $44.4\% \pm 1.0\%$ at 100% $\dot{V}O_{2peak}$ in EU and AM, respectively). Cortisol levels were above baseline in both groups for all exercise

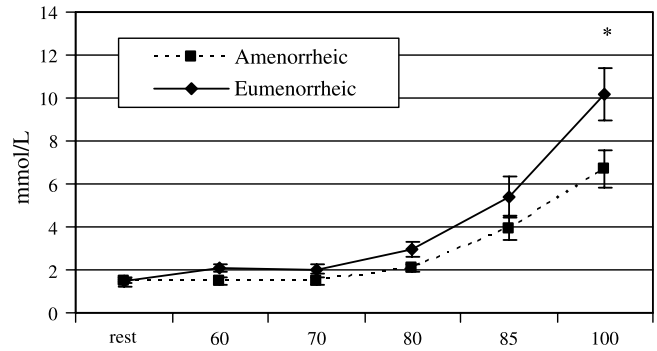


FIGURE 2—Blood lactate concentration at rest and during exercise. *Significantly different from AM, $P \leq 0.05$.

intensities except 85% and 100% of $\dot{V}O_{2peak}$, with no differences between groups ($P > 0.3$).

AM had 34% lower lactate (Fig. 2) values at peak exercise ($P = 0.01$). Lactate rose above resting concentrations at 80% and 85% of $\dot{V}O_{2peak}$ in both EU and AM, respectively. Epinephrine (Fig. 3) levels did not change from rest through submaximal exercise and were not significantly different between groups. Epinephrine rose sharply at $\dot{V}O_{2peak}$ in the EU group and was 3.25 times greater in EU than that in AM ($P = 0.02$). At 70% of $\dot{V}O_{2peak}$, norepinephrine (Fig. 4) rose significantly from rest, increasing further with each workload until $\dot{V}O_{2peak}$. From 70% to maximal workload, EU showed a greater norepinephrine response than AM ($P = 0.03$). Peak norepinephrine concentrations of AM were only half that of EU ($P = 0.0002$).

Table 6 describes the correlations between peak lactate and catecholamines concerning menstrual status. All three variables were significantly correlated with ExEE per lean body mass, the percentage of exercise as total energy

Table 5. Exercise cardiovascular and blood parameters.

Variable		Exercise Intensity as % $\dot{V}O_{2peak}$				
		60%	70%	80%	85%	100%
Speed (km·h ⁻¹)	EU	12 ± 0.4	12 ± 0.4	14 ± 0.5	14 ± 0.4	17 ± 0.5
	AM	10 ± 0.3	12 ± 0.4	13 ± 0.4	14 ± 0.4	16 ± 0.6
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	EU	35 ± 1.0	41 ± 1.1	47 ± 2.0	50 ± 1.9	57 ± 2.3
	AM	34 ± 1.2	39 ± 1.5	45 ± 2.0	48 ± 1.8	56 ± 1.2
Actual % of $\dot{V}O_{2peak}$	EU	62 ± 1.5	72 ± 1.3	82 ± 1.5	87 ± 1.0	100 ± 0.0
	AM	60 ± 0.7	69 ± 1.5	79 ± 1.9	86 ± 1.7	100 ± 0.0
RER	EU	0.85 ± 0.02	0.91 ± 0.01 ^a	0.94 ± 0.01 ^{a,b}	0.98 ± 0.00 ^{a,b}	1.13 ± 0.00 ^{a,b,c,d}
	AM	0.87 ± 0.02	0.9 ± 0.01 ^a	0.94 ± 0.01 ^a	0.96 ± 0.02 ^{a,b}	1.13 ± 0.02 ^{a,b,c,d}
RPE (scale 0–10)	EU	2.2 ± 0.4	3.6 ± 0.2	5.6 ± 0.0	7.9 ± 0.8	9.9 ± 0.1
	AM	2.2 ± 0.4	3.9 ± 0.5	5.6 ± 0.7	7.6 ± 1.0	10.0 ± 0.0
HR (bpm)	EU	150 ± 6.2	160 ± 4.0	180 ± 4.0	190 ± 3.6	200 ± 3.1
	AM	140 ± 4.1	160 ± 6.5	170 ± 6.2	190 ± 6.9	190 ± 5.7
MAP (mm Hg)	EU	91 ± 2.1	92 ± 2.0	95 ± 4.1	95 ± 4.1	100 ± 1.7 ^{a,b}
	AM	89 ± 2.6	91 ± 2.5	94 ± 4.9	95 ± 4.0	100 ± 2.8 ^{a,b,c}
Glucose (mmol·L ⁻¹)	EU	5.5 ± 0.5	4.8 ± 0.4 ^a	5.1 ± 0.3	7.1 ± 0.6 ^{a,b,c}	7.1 ± 0.4 ^{a,b,c}
	AM	5.1 ± 0.1	4.6 ± 0.2	5.1 ± 0.1	6.9 ± 0.7 ^{a,b,c}	6.3 ± 0.5 ^{a,b,c}
Cortisol (μg·dL ⁻¹)	EU	19 ± 2.2	19 ± 3.5	17 ± 2.7	14 ± 2.6 ^{a,b,c}	11 ± 1.9 ^{a,b,c,d}
	AM	21 ± 1.3	20 ± 1.4	18 ± 1.4	17 ± 1.4 ^{a,b}	15 ± 2.0 ^{a,b,c}

Values are presented as mean ± SEM for five EU and five AM women.

Unless indicated, for each variables, values significantly increased at each intensity.

* Significantly different from EU; $P \leq 0.05$.

^a Significantly different from 60% $\dot{V}O_{2peak}$.

^b Significantly different from 70% $\dot{V}O_{2peak}$.

^c Significantly different from 80% $\dot{V}O_{2peak}$.

^d Significantly different from 85% $\dot{V}O_{2peak}$.

$\dot{V}O_2$, oxygen consumption; MAP, mean arterial pressure.



FIGURE 3—Plasma epinephrine concentrations during rest and exercise. *Significantly different from AM, $P \leq 0.05$.

expenditure and EA. Both catecholamines were also significantly correlated with menstrual cycles per year. Peak lactate significantly correlated with both peak catecholamines. When these variables were added to a stepwise regression, only the percentage of exercise to total energy expenditure entered the model and accounted for 57% of the variance in the peak epinephrine response. Both menstrual cycles per year and EA entered the model for peak norepinephrine accounting for 85% of the variance in the peak norepinephrine response. Only EA entered the model for peak lactate and accounted for 65% of the variance in the peak lactate response.

DISCUSSION

This experiment is the first that we know of to determine the relationship between the menstrual status and the plasma catecholamine response to maximal exercise. We found a reduced catecholamine response to high-intensity exercise in AM athletes compared with normal menstruating (EU) controls matched for age, years of training, and self-reported running kilometers per week. This blunted peak epinephrine and norepinephrine response was correlated with lower peak blood lactate, higher levels of ExEE as a percentage of total energy expenditure, lower EA, and fewer menstrual cycles per year.

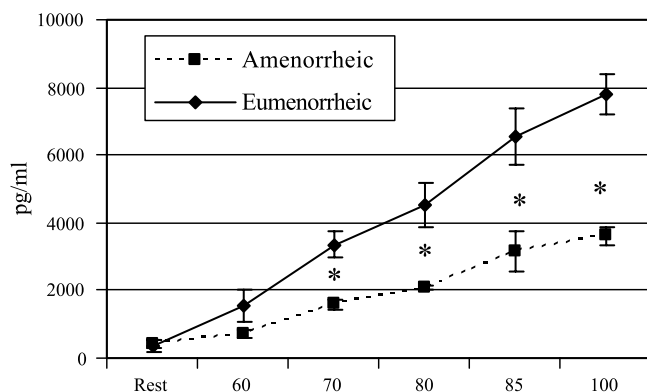


FIGURE 4—Plasma norepinephrine concentrations during rest and exercise. *Significantly different from AM, $P \leq 0.05$.

Table 6. Correlations between energy status, peak catecholamines, and peak lactate.

Variable	R^2	P Value	Intercept	Coefficient
Epinephrine (pg·mL ⁻¹)			2322.3	
Menstrual cycles per year	0.49	0.020		
Exercise EE (kcal·kg ⁻¹ LBM·d ⁻¹)	0.46	0.030		
% total EE as exercise	0.57	0.010		-35.82
EA (kcal·kg ⁻¹ LBM·d ⁻¹)	0.45	0.030		
Norepinephrine (pg·mL ⁻¹)			2189.00	
Menstrual cycles per year	0.68	0.004		258.01
Exercise EE (kcal·kg ⁻¹ LBM·d ⁻¹)	0.42	0.040		
% total EE as exercise	0.54	0.020		78.30
EA (kcal·kg ⁻¹ LBM·d ⁻¹)	0.42	0.040		
Lactate (mmol·L ⁻¹)			4.37	
Exercise EE (kcal·kg ⁻¹ LBM·d ⁻¹)	0.56	0.010		
% total EE as exercise	0.59	0.009		
EA (kcal·kg ⁻¹ LBM·d ⁻¹)	0.65	0.005		0.17
Peak epinephrine (pg·mL ⁻¹)	0.61	0.01		
Peak norepinephrine (pg·mL ⁻¹)	0.61	0.01		

Intercepts and regression coefficients from stepwise regression.
EE, energy expenditure; LBM, lean body mass.

AM and EU subjects were successfully matched by age and training volume. Groups were similar in height, weight, and body composition. This is in contrast to a recent study by Christo et al. (3) in which similar lean body mass was found in athletes aged 12–18 yr, but the AM subjects had significantly lower body weights and fat mass. However, these athletes were not matched according to training volume, which could explain the differences between studies. Rickenlund et al. (39) examined body composition in athletes matched for training hours per week and found no differences in body weight, lean mass, or fat mass between AM and EU athletes. It has been suggested that the essential body fat necessary for normal reproductive function in women is 17%–22% (14). However, growing evidence is supporting the idea that low body fat percentage is not always associated with amenorrhea but is instead a consequence of low EA (27).

The loss of menstrual cycles in AM women suggests long-term energy insufficiency, possibly stemming from a continuous failure, intentional or not, to match caloric intake to energy expenditure. EA is defined in our study as dietary EI minus ExEE or the amount of dietary energy remaining for other body functions after exercise training (31). Adaptive mechanisms exist, which enable the suppression of nonessential metabolic processes under conditions of low EA, conserving vital fuel stores for individual survival rather than maintaining reproductive fitness (47). Although studies have found an elevated occurrence of disordered eating patterns and amenorrhea in women involved in aesthetic and endurance disciplines (19,48), none of our subjects suffered from eating disorders like anorexia nervosa or bulimia nervosa. This was confirmed by their physician before subjects entered the study and was confirmed by scores within the normal range for the EDE-Q questionnaire. However, the AM women scored significantly higher than the EU women on the EDE-Q's global score and shape concern score subscales. On the POMS questionnaire, our subjects' results were similar to those of athletes studied by Cockerill et al. (4) in that all the negative mood states were lower and

the vigor higher than that in the age-matched sedentary controls. However, in the study by Cockerill et al. (4), AM athletes showed higher mood disturbances despite similar vigor scores. Our POMS data were similar to that of Loucks and Horvath (28) because we were not able to distinguish alterations in mood or fatigue in our AM subjects, which could have helped explain their altered catecholamine response to exercise.

We did not find any significant difference in EA on the basis of menstrual status in our study. The AM athletes as a group did fall below $20 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day, however, and LH pulsatility has been shown to be disrupted within 5 d when the EA of young women was reduced by more than 33% from 45 to less than $30 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day (31) and restored when EA increased from an average of $25\text{--}30 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day. Some women may be more susceptible to the effects of low EA than others because we had one AM subject that had not menstruated for 2 yr but had an EA of $36 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day and two EU subjects with an EA below $30 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day. EA may fluctuate above and below the $30 \text{ kcal} \cdot \text{kg}^{-1}$ of lean body mass per day depending on training volume and intensity and may affect menstrual function only when consistently low. It is possible that although our EU subjects were menstruating regularly, they could have had subclinical menstrual disorders such as luteal phase deficiency and anovulation related to lower EA. We did find that the percentage of energy expenditure from exercise was higher in AM athletes.

Adaptive mechanisms that suppress nonessential processes under conditions of low EA may also affect other physiological systems to conserve vital fuel stores. Catecholamines released by the sympathetic nervous system and the adrenal medulla assist with blood glucose regulation and exercise fuel use by mobilizing liver and muscle glycogen. Pirke (38) found reduced sympathetic nervous system activity with starvation and restricted eating and lower peak exercise norepinephrine values in anorexic patients. Under resting conditions, AM subjects in our study showed a lower HR and MAP than EU subjects. Although bradycardia from attenuated sympathetic activity and increased vagal tone is a well-known adaptation to endurance training (26), differing fitness levels between the two groups was likely not the cause for these observations. Subjects were carefully matched by training status, and AM subjects reached slightly lower $\dot{V}O_{2\text{peak}}$ values than their EU matches. Our results agree with the findings of O'Donnell et al. (37), who found significantly lower resting HR and systolic BP in AM athletes.

Although running speeds and corresponding rates of oxygen consumption were similar between the two groups at all submaximal stages, the catecholamine response to exercise differed markedly. As early as at 70% of $\dot{V}O_{2\text{peak}}$, AM athletes displayed a significantly lower norepinephrine concentration than EU athletes, and the magnitude of this difference increased until $\dot{V}O_{2\text{peak}}$, where norepinephrine levels in EU women were twice as high as those of their AM

matches. Epinephrine levels did not rise in either group until $\dot{V}O_{2\text{peak}}$, at which point they were three times higher in EU women. We found only one study that examined the catecholamine response to exercise between AM versus EU athletes. Loucks and Horvath (28) did not find a difference in catecholamine levels between AM and EU athletes, but blood samples were drawn 4 min after exercise at 85% $\dot{V}O_{2\text{max}}$, well beyond the 1- to 2-min half-life for catecholamines. Our blood samples were drawn immediately after exercise. They also did not investigate the neuroendocrine response at peak exercise capacity, which could explain why they did not find a difference in epinephrine concentrations because we only saw a difference at $\dot{V}O_{2\text{peak}}$.

Norepinephrine and epinephrine play crucial roles during intense exercise, ensuring adequate metabolic and cardiovascular responses to the imposed workload. As the intensity of exercise rises above 50% or 60% of $\dot{V}O_{2\text{peak}}$, the centrally mediated increase in sympathetic activation causes catecholamine concentrations to rise significantly (2). The rising norepinephrine concentration from synaptic spillover stimulates hepatic glucose production and is therefore associated with the sharp increase in glucose appearance rates as exercise intensity rises (2,21). Epinephrine, secreted from the adrenal medulla, stimulates muscle glycogenolysis and lactate production, thereby supporting muscle metabolism and gluconeogenesis. As work intensity rises, glycolysis takes on an increasingly important role as ATP supplier, supplementing the slower oxidative metabolic process to fuel the working muscle fibers (2). Catecholamine levels, therefore, rise steadily to maintain euglycemia. At maximal effort, norepinephrine and epinephrine concentrations peak, typically causing a large glucose spill into the circulation (21) and allowing peak glycolytic rates to be reached. This is also evidenced by the exponential accumulation of blood lactate, the product of glycolysis, with rising workload.

Lactate levels were significantly lower at $\dot{V}O_{2\text{peak}}$ in AM than that in EU subjects. Although we were not able to directly measure the rates of carbohydrate and fat utilization, these reduced lactate concentrations could be explained by the reduced sympathetic drive in AM women, resulting in a reduced glycolytic response at maximal exercise intensity. It should be mentioned that the pattern and the magnitude of the catecholamine response and subsequently relative fuel utilization rates at maximal exercise are affected by energy status and physical fitness (2,13,21). It has been shown that the increase in $\dot{V}O_{2\text{peak}}$ and maximal workload attained by individuals after training is accompanied by a higher peak catecholamine response (2,13,21) with a greater glucose spill and lactate accumulation at $\dot{V}O_{2\text{peak}}$ (21). Given the similarity of the training patterns of the two groups in this study and their running performances in the laboratory, the two- to threefold differences in peak catecholamine levels could not be attributed to fitness discrepancies. Instead, we speculate that a blunted catecholamine response could represent an adaptation that promotes survival during chronic energy deficiency. Indeed, as exercise provides an additional

challenge to energy homeostasis, a suppressed capacity for maximal catecholamine stimulation would keep energy-deficient individuals from depleting limited glycogen stores at a fast rate, preserving glucose for vital organ functions. Blunted sympathoadrenal responses have been documented in food-deprived individuals (23) as well as in chronically overtrained athletes (44). Blunted sympathoadrenal responses in overtrained female athletes likely comes as a result of low estrogen levels from frequent glycogen-depleting training sessions without adequate EI (46). Further, despite similar caloric and carbohydrate intake before the test, AM women exhibited a trend ($P = 0.1$) toward lower resting blood glucose levels, a finding that agrees with that of Laughlin and Yen (24). Because lower blood glucose levels are seen in AM versus EU (24), we applied a one-sided unpaired t -test and yielded a significantly lower glucose levels in AM ($P = 0.05$). The mild hypoglycemia reported by other studies (24,32) in AM women reveals a state of energy deficiency, as do the other endocrine-metabolic aberrations seen in this population, such as hypoinsulinemia (24,41), hypothyroidism (24,29), hypercortisolemia (7,8,24), and reduced basal metabolic rate (25,41).

Low EA has been shown to affect several aspects of the hypothalamic–pituitary axis. Acting as the principal catabolic hormone, cortisol is secreted by the adrenal cortex in response to prolonged exercise, starvation, or glycogen depletion (33). Hypercortisolism can therefore be observed in undernourished or glycogen-depleted athletes (45) because food deprivation adds to the already high metabolic demand of training. Loucks and Horvath (28) found well-trained female athletes to exhibit higher early-morning cortisol levels than sedentary individuals, despite similar 24-h ACTH pulse patterns and cortisol pulse frequencies. Further, AM athletes in this same study maintained this mild hypercortisolism through the morning and evening. Although Kanaley et al. (20) found elevated resting and exercise (90 min at 60% $\dot{V}O_{2peak}$) cortisol levels in AM versus EU athletes, controversy exists. De Souza et al. (7,8) found that despite mild hypercortisolism at rest, AM athletes displayed a blunted cortisol response to maximal and submaximal exercise, possibly because of a reduced adrenal sensitivity to ACTH as a result of chronically elevated cortisol. Although we did not find a difference in resting or exercise cortisol concentrations in our subjects, the difference in results could be a result of methodology. Our subjects were tested 2–3 h after a meal and in the morning, whereas the subjects in the study of De Souza et al. (7,8) were tested over 6 h after a meal and in the afternoon. Our submaximal bout (between 60% and 85% $\dot{V}O_{2peak}$) of exercise lasted 27 min versus 40 min at 80% $\dot{V}O_{2peak}$ in the study of De Souza et al. (7,8). As cortisol displays a circadian rhythm, the timing of the exercise measurements would be important, with the highest levels (and the most rapid decline over time) seen in the morning and the lowest levels in the evening (42).

When men are exposed to elevated cortisol levels, induced by either hypoglycemia or exercise, their neuroen-

docrine and metabolic responses to subsequent hypoglycemia or exercise are blunted (17). This phenomenon, known as HAAF, is not caused by hypoglycemia *per se* but rather by the binding of cortisol to corticosteroid receptors in the brain during the antecedent event (18). HAAF persists as long as cortisol remains bound to these receptors, which may be several days (17). The fact that HAAF does not occur in healthy young women may be explained by estrogen blocking cortisol from binding to these receptors (12,43). Estrogen replacement blunts catecholamine responses to hypoglycemia in postmenopausal women (40), but to our knowledge, the influence of estrogen on the catecholamine response after an antecedent elevation in cortisol (i.e., HAAF) has not been determined by comparing women with normal and low estrogen levels. Although this hypothesis has not yet been tested, the smaller catecholamine responses to exercise in our AM subjects might be explained by the ability of cortisol to bind to the corticosteroid receptors during exercise training in the week before data collection because of low estrogen concentrations. This interpretation is supported by a smaller epinephrine response to 90 min of exercise at 50% of $\dot{V}O_{2max}$ in healthy young men than that in healthy young women after a similar bout of exercise earlier in the same day (15). However, we cannot be sure that estrogen-modulated HAAF accounted for our results, because we cannot be sure that estrogen in our EU subjects, tested during the early follicular phase of the menstrual cycle, was higher than that in our AM subjects over the days leading up to the testing sessions.

It could be argued that despite developing amenorrhea, these athletes may also have become more competitive runners as a result of intense training. However, we suspect that their current performances might still improve if the energy deficiency responsible for this condition was corrected. Williams et al. (49) showed that FHA could be reversed within 12–57 d in highly active monkeys after the recovery of adequate energy balance, indicating that normal hypothalamic–pituitary–gonadal activity can recover relatively quickly. The same appears to hold true for the other hypothalamic–pituitary axes because athletes have recovered normal cortisol and thyroid hormone levels after recovering from anorexia and amenorrhea (11). These findings encourage the notion that normal sympathoadrenal activation might also be recovered as a result of improved EA. In addition, the catecholamine and the lactate responses to maximal exercise may have the potential to be used as biomarkers for adequate EI and estrogen levels in competitive female athletes. In our group of athletes, all AM subjects had peak epinephrine values below $550 \text{ pg}\cdot\text{mL}^{-1}$ and peak norepinephrine values below $4450 \text{ pg}\cdot\text{mL}^{-1}$, and four of five subjects had peak lactates below $8 \text{ mmol}\cdot\text{L}^{-1}$. In comparison, all EU subjects had epinephrine values above $750 \text{ pg}\cdot\text{mL}^{-1}$ and norepinephrine values above $6100 \text{ pg}\cdot\text{mL}^{-1}$, and four of five subjects had peak lactates above $8 \text{ mmol}\cdot\text{L}^{-1}$.

To our knowledge, this is the first study to examine the sympathetic response to maximal exercise in carefully

matched AM and normally menstruating athletes. The sample size for this study was small, and activity logs and activity monitors may not have accurately measured ExEE. We may have seen significant differences in many of the variables with a larger sample size. We recognize that longitudinal investigations using a more precise measure of ExEE such as doubly labeled water and thorough measurements of reproductive status such as serum estradiol, progesterone, follicle stimulating hormone, and luteinizing hormone will be needed to determine how both the development of and the recovery from energy deprivation-related FHA may affect the catecholamine response to exercise.

This study revealed significantly impaired catecholamine response to maximal and submaximal exercise in athletes with hypothalamic amenorrhea, reflected in lower peak lactate concentrations. These findings add to the quickly expanding base of knowledge regarding the adverse health

effects of energy deficiency and hypoenestrogenism. Women diagnosed with FHA should be thoroughly educated on the cause and adverse health consequences of this condition to fully recognize the importance of restoring optimal EA.

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