Preserved autonomic function in amenorrheic athletes

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Preserved autonomic function in amenorrheic athletes. J Appl Physiol 101: 590–597, 2006. First published May 18, 2006; doi:10.1152/japplphysiol.01477.2005.—Reproductive hormones such as estradiol and progesterone are known to influence autonomic cardiovascular regulation. The purpose of this study was to determine whether amenorrheic athletes (AA) have impaired autonomic cardiovascular regulation compared with eumenorrheic athletes (EA). Thirty-five athletes were tested: 13 AA (19 ± 1 yr), 13 EA (21 ± 1 yr), and 9 EA (23 ± 1 yr) on oral contraceptives (EA-OC).

Multiple indexes of autonomic cardiovascular regulation were assessed: respiratory sinus arrhythmia (RSA), baroreflex sensitivity (BRS) via phase IV and phase II of the Valsalva maneuver, a spontaneous index of BRS, and the heart rate and blood pressure sensitivity (BRS) via phase IV and phase II of the Valsalva maneuver, assessed: respiratory sinus arrhythmia (RSA), cardiovagal baroreflex sensitivity (BRS) via phase IV and phase II of the Valsalva maneuver, and a spontaneous index of BRS. RSA was not a spontaneous index of BRS, and the heart rate and blood pressure sensitivity (BRS) via phase IV and phase II of the Valsalva maneuver, assessed: respiratory sinus arrhythmia (RSA), cardiovagal baroreflex sensitivity (BRS) via phase IV and phase II of the Valsalva maneuver, and a spontaneous index of BRS.

To the best of our knowledge, no studies have examined autonomic cardiovascular regulation in AA. Accordingly, the purpose of this study was to assess several indexes of cardiovagal BRS and several indexes of vagal tone in AA. We hypothesized that amenorrheic athletes would have low cardiovagal BRS and vagal tone. To test this hypothesis, we compared a group of AA (low reproductive hormones all the time) with two distinct groups of eumenorrheic athletes (EA), one group not taking oral contraceptives (fluctuating reproductive hormones) and one group taking oral contraceptives (EA-OC; high reproductive hormones most of the time). Our rationale for this comparison was that AA are not exposed to cyclic variations in reproductive hormones, whereas EA and EA-OC are exposed to varying degrees of endogenous and/or exogenous hormones during the course of a normal menstrual cycle. Thus the purpose was to determine whether autonomic function was lower from the chronic suppression of reproductive hormones in the AA group. Because the autonomic nervous system regulates blood pressure and heart rate (HR) responses to changes in posture, a tilt-table test was used to measure cardiovascular responses to orthostatic stress. Impaired autonomic cardiovascular regulation may be associated with orthostatic intolerance (4); therefore, we hypothesized that AA would have attenuated cardiovascular responses to a head-up tilt compared with the two groups of EA.

MATERIALS AND METHODS

Subjects

Thirty-six healthy women were recruited for this study. Fourteen AA (secondary amenorrhea is defined as the absence of three or more menstrual cycles in the preceding year) and 22 EA (normal menstrual cycles) were recruited.

FEMALE REPRODUCTIVE HORMONES fluctuate throughout the menstrual cycle and are known to influence autonomic cardiovascular regulation (14, 20, 21). These hormones are released in a cyclic fashion and are controlled by the hypothalamus (19).

The proposed mechanism underlying menstrual disturbances is a disruption in the release of gonadotropin-releasing hormone from the anterior pituitary. The hypothalamic-pituitary-gonadal system regulates blood pressure and heart rate (HR) responses to changes in posture, a tilt-table test was used to measure cardiovascular responses to orthostatic stress. Impaired autonomic cardiovascular regulation may be associated with orthostatic intolerance (4); therefore, we hypothesized that AA would have attenuated cardiovascular responses to a head-up tilt compared with the two groups of EA.

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Both acute intravenous and subcutaneous administration of 17β-estradiol improved baroreflex function in ovariectomized female rats (24, 29). Chronic subcutaneous administration of 17β-estradiol also improved baroreflex function in ovariecto-
consecutive menstrual cycles after menarche), 13 EA (self-reported normal menstrual cycle ~28 days), and 9 EA-OC were recruited to participate in the study. The length of amenorrhea ranged from 3 mo to 1 yr in subjects recruited for the study. The mean maximal oxygen consumption (\(V_\text{O}_2\text{max}\)) value of each group was greater than the 90th percentile for their age range based on the American College of Sports Medicine’s Guidelines for Exercise Testing and Prescription handbook (10). Subjects consisted of competitive and recreational runners (n = 27), figure skaters (n = 6), a cyclist (n = 1), and rowers (n = 2). The oral contraceptive regimen varied among the subjects (information documented in 7 of 9 subjects) in the EA-OC group (Ortho Evra, Estrostep, Micrette, and Triphasic regimens). Reasons for using oral contraceptives included birth control, correcting menstrual irregularities, and decreasing menstrual flow. Subjects were excluded from the study if they were pregnant, were experiencing oligomenorrhea, or had primary amenorrhea. An ovulation prediction kit (Clearblue Easy ovulation test by Unipath Diagnostics, Waltham, MA) was used for normal-menstruating EA not on oral contraceptives to rule out anovulation. All female subjects were between the ages of 16 and 30 yr, nonsmokers, and not taking any medications for at least 6 mo. One amenorrheic subject was excluded from the study because she resumed menstruation 1 wk after the testing. Therefore, 35 subjects were used for the analysis: 13 AA, 13 EA, and 9 EA-OC. The study was approved by the University of Delaware Human Subjects Review Board. Written and verbal informed consent was received from all subjects.

**Screening Visit**

The study required that the participants attend two 2-h sessions at the University of Delaware’s Human Performance Laboratory. Each subject filled out a medical history questionnaire and physical activity readiness questionnaire. All subjects abstained from food, caffeine, alcohol, and exercise for 12 h before the first testing session. A baseline blood sample was obtained for a complete blood count, fasting glucose, liver function (i.e., aspartate transaminase and alanine transaminase), kidney function (i.e., creatinine and blood urea nitrogen), albumin, and electrolytes (i.e., sodium, potassium, and chloride) to ensure all subjects were not displaying signs of renal or hepatic disease or of an electrolyte imbalance. Height and weight were measured (Healthometer scale, Continental Scale, Bridgeview, IL). A 12-lead ECG (Schiller AT-10, Electro-Med, Flint, MI) was completed at rest and during an exercise test. A running protocol was used during the exercise test, where the speed was self-selected (between 5 and 7 mph/h). The grade started at 0% and was increased 2% every 2 min during the test. Expired air was collected for the analysis of oxygen, carbon dioxide, and volume to determine each subject’s \(V_\text{O}_2\text{max}\) (TrueOne 2400 Metabolic Measurement System, ParvoMedics, Sandy, UT). The test was considered a maximal test if at least two of the following criteria were met: 1) >85% age predicted maximal HR, 2) rating of perceived exertion >17 on a 6–20 Borg scale, or 3) respiratory exchange ratio >1.10. Bone density and body composition were determined for each subject using a dual-energy X-ray absorptiometry Hollogic whole body scan (version 3.6y Lunar, Madison WI). Each subject was asked to keep a 3-day dietary food record while wearing an Actical accelerometer (Mini Mitter, Bend, OR) over the left hip to assess energy intake and energy expenditure. The caloric intake was analyzed using the US Department of Agriculture website.

**Experimental Visit**

Cardiovagal baroreflex sensitivity (BRS), vagal tone, and cardiovascular responses to orthostatic stress were assessed during the second visit. Each EA and EA-OC subject reported to the laboratory during the early follicular phase (~3–5 days after the onset of menstruation, which was also during the placebo phase of oral contraceptive use) to perform the tests. Our intent was to determine whether chronic (i.e., 3 mo or longer) low estradiol affected BRS. At the point of testing, both EA and AA groups should have low estradiol and progesterone, and therefore they were similar based on hormonal concentration; the difference is that the AA group has low hormone levels all the time, whereas the EA group has low hormones for ~5 days of each menstrual cycle. In contrast, EA-OC have high levels of estradiol and progesterone most of the time (taking exogenous hormones). All subjects abstained from alcohol, caffeine, and exercise for 12 h and from food for 4 h before the second testing session. A blood sample was collected and stored at ~70°C for future analysis of 17β-estradiol, progesterone, total 3,5,3′-triiodothyronine (T3), and thyroid-stimulating hormone (TSH). Assays for 17β-estradiol and progesterone were performed on the Roche Elecsys 2010. The intra- and interassay coefficients of variation for 17β-estradiol ranged from 1.6 to 5.7% and from 2.3 to 6.2%, respectively, in the measurement range of 5–4,300 pg/ml. The intra- and interassay coefficients of variation for progesterone ranged from 1.7 to 2.7% and from 3.7 to 5.4%, respectively, in the measurement range of 0.030–60.00 pg/ml.

**Protocol**

**Measurements.** **BLOOD PRESSURE, HR, AND RESPIRATION.** Blood pressure was measured using the Dinamap Dash 2000 (GE Medical Systems). During testing procedures, beat-by-beat arterial blood pressure was measured using a Finometer (Finapres Medical System, Arnhem, The Netherlands) worn on the finger, which was calibrated using the manufacturer’s return-to-flow calibration. The Finometer is a reliable, noninvasive technique to quantify and track arterial blood pressure at rest and during autonomic cardiovascular testing (15). Respiration rate was also measured during the test, using the Inductotrace (Ambulatory Monitoring, Ardsley, NY), which consisted of two elastic bands that are placed around the rib cage and abdomen. HR was measured using a single-lead ECG through the Dinamap Dash 2000; this was used to calculate R-R interval.

**ASSESSMENT OF VAGAL TONE.** R-R interval length varies during normal inspiration and expiration. This variability is known as respiratory sinus arrhythmia (RSA), and it is an index of cardiac vagal tone (6). RSA was measured during 5 min of paced breathing at 0.25 Hz (15 breaths/min) for two trials. Paced breathing was used to avoid changes in breathing frequency that can influence the magnitude of RSA. Respiration, R-R interval, and beat-by-beat blood pressure were recorded during the paced breathing. See Data Analysis for additional details on quantifying RSA.

R-R variation during deep breathing (6 breaths/min) and the Valsalva maneuver were assessed by the ANS 2000 ECG Monitor and Respiration Pacer (D. E. Hokanson, Bellevue, WA). R-R variation was quantified by vector analysis after 6 min of deep breathing and reported as the mean circular resultant (MCR) (33). The MCR is independent of intrinsic HR and is less sensitive to premature beats compared with other methods of assessing R-R variation (33). HR responses to the Valsalva maneuver were determined by having the subjects expire into a mouthpiece, maintaining a pressure of 40 mmHg for 15 s. During the maneuver, tachycardia and peripheral vasoconstriction develop during strain, and there is bradycardia and an overshoot in blood pressure during the release. The ratio of the longest R-R interval after the maneuver to the shortest R-R interval during the maneuver is referred to as the Valsalva ratio, and it is used in the clinical literature. R-R variation is thought to be a function of parasympathetic activity (26), whereas the Valsalva ratio is thought to be a more generalized index of autonomic nerve function. Pharmacological blocking studies (30) suggest that the Valsalva ratio is influenced mainly by parasympathetic but also by sympathetic activity (11, 32).

**ASSESSMENT OF CARDIOVAGAL BRS.** Cardiovagal BRS was assessed using a separate Valsalva maneuver, where the strain causes an increase in intrathoracic and intra-abdominal pressure, causing an initial fall in blood pressure (phase II) and subsequent rise in pressure above baseline (phase IV). The change in pressure relative to the R-R...
INTERVAL (i.e., the slope of the relationship) is a robust index of cardiovagal BRS that correlates with more invasive techniques (27). Both phase II (pressure decline and reflex tachycardia) and phase IV (pressure overshoot and reflex bradycardia) were used as separate indexes of cardiovagal BRS (23). Each subject was instructed to inhale maximally and then breathe into a tube connected to a pressure transducer so that the expiratory pressure was maintained at 40 mmHg. The subjects maintained this pressure for 15 s (Fig. 1). Arterial blood pressure and R-R interval (via ECG) were measured during this time and immediately after the termination of the maneuver. The Valsalva maneuver was performed in triplicate. Between each Valsalva maneuver, there was a minimum rest period of 5 min to allow HR and blood pressure to return to resting levels. Reliability pilot studies conducted in our laboratory on four subjects over 2 different days for the Valsalva maneuver resulted in a coefficient of variation of 11.8%.

A time-domain sequential technique was used to calculate a spontaneous index of BRS by detecting a linear change in blood pressure and pulse interval for at least four beats in the same direction (25). Blood pressure data were downloaded from the Finometer using the BeatScope software program (Finapres Medical System). A DOS-based program (available from the same company) was used to calculate the spontaneous index of BRS. This technique has been shown to correlate with the vasoactive drug technique (25). Thus three separate indexes of cardiovagal BRS were used (phase IV of the Valsalva, phase II of the Valsalva, and a spontaneous index of BRS).

CARDIOVASCULAR RESPONSES TO ORTHOSTATIC STRESS. Respiration, HR, and beat-by-beat blood pressure were measured at rest and during a 20-min 60°head-up tilt. Venous occlusion plethysmography was used to assess limb (i.e., arm) vascular resistance during the tilt, providing an index of sympathetic peripheral vasoconstriction (37). While the subjects were supine, a mercury-in-Silastic strain gauge was placed around the largest portion of the lower arm, a large blood pressure cuff around the upper arm, and a small cuff around the wrist (to transiently occlude blood flow to the wrist). The wrist cuff was inflated to suprasystolic blood pressure levels (i.e., 200 mmHg), and the upper arm cuff was rapidly inflated (model E20 rapid cuff inflator, D. E. Hokanson, Bellevue, WA) to ~55 mmHg (above venous pressure but below diastolic pressure) for 8 s, allowing arterial blood into the limb but temporarily preventing venous blood from escaping. The calibrated strain gauge around the arm was connected to a plethysmograph (model EC6, D. E. Hokanson), which records the circumferential change in the arm. The relative increase in the circumference of the strain gauge was recorded (the slope of change in limb volume was plotted as a function of time) providing an index of arterial flow into the limb, and is expressed as milliliter per 100 milliliter of limb tissue per minute. The upper arm cuff was deflated for a period of 7 s; therefore, an index of limb blood flow (and resistance) was determined every 15 s. Two minutes of data were collected for each time point. Limb vascular resistance was calculated as the mean arterial pressure (MAP; derived from the Finometer) divided by arm blood flow and is expressed in arbitrary units. All signs and symptoms were closely monitored and recorded. The tilt test was considered abnormal if the subjects 1) experienced syncope or presyncope or symptoms (e.g., lightheadedness, nausea, etc.), 2) experienced a significant fall in blood pressure, and 3) had an abnormal HR response (i.e., bradycardia associated with vasovagal syncope).

**Data Analysis**

Beat-by-beat blood pressure, ECG, and respiration were collected at 500 Hz using Windaq software (DATAQ Instruments, Akron, OH). To quantify RSA, power spectral analysis was performed on the 300 s R-R interval time series. Data were analyzed using signal-processing software (CODAS, DATAQ Instruments) to peak detect each R-wave from the QRS complex and to peak and valley detect the blood pressure signal. The data were detrended and analyzed with a fast Fourier transform, and the area under the curve within the respiratory frequency band, defined as 0.2–0.3 Hz, was summed to generate an estimate of RSA/vagal tone (35).

Linear regression analysis was used to relate systolic blood pressure and R-R interval during phase II and phase IV of the Valsalva maneuver, with an R-R interval lag of 1 (Fig. 2) illustrates an example of regression analysis derived from phase IV. Only regressions with an \( r^2 > 0.7 \) were included in the analysis. On average, there were...
8–10 points included in the regression for phase II and 4–6 points for phase IV. Although each subject performed three trials, not all three trials were averaged. Based on the r value, some trials were not utilized. Most of the subjects had an average of two trials with an \( r^2 > 0.80 \).

HR and MAP were averaged over 2-min periods at baseline and four time points during the 20-min tilt. Limb vascular resistance was calculated at baseline and every 5 min during tilt by dividing MAP by forearm blood flow.

**Statistics**

One-way ANOVAs were used to determine differences in cardiovagal BRS and RSA among the three groups. If simple main effects were found, post hoc analyses were performed with a Tukey’s correction. When appropriate, age was used as a covariate in the analysis. A two-way repeated measures ANOVA was used to assess tilt responses in AA, EA, and EA-OC. Post hoc pairwise comparisons for differences across time during the tilt were performed using estimated marginal means. Power calculations indicated we had 80% power to determine an effect size of 0.55. All data are presented as means ± SE.

**RESULTS**

**Subject Characteristics**

Subject characteristics are presented in Table 1. There were no differences among the groups with regards to height, weight, \( \dot{V}O_2 \text{max} \), percent body fat, and bone mineral density (BMD). AA were slightly younger than EA-OC. All baseline blood work was within clinically acceptable normal limits for all groups, except one AA subject had a lower than normal hemoglobin of 10.5 g/dl. There were no differences in hematocrit, hemoglobin, red blood cell count, glucose, sodium, potassium, chloride, creatinine, calcium, blood urea nitrogen, aspartate transaminase, or alanine transaminase among the groups. AA had a higher albumin level compared with EA-OC (AA = 4.9 ± 0.1 g/dl vs. EA-OC = 4.4 ± 0.1 g/dl; \( P = 0.02 \)). Reproductive hormone measurements were collected on 29 of the 35 subjects. The data are presented in Table 2. Progesterone was lower in the EA-OC group compared with the AA and EA groups (\( P < 0.05 \)). Total T2 was higher in the EA-OC group compared with the AA and EA groups (\( P < 0.01 \)). There were no differences in 17β-estradiol and TSH among the groups.

Data from the 3-day dietary food record and daily expenditure were averaged for each group. There were no differences in the average daily intake among each group (AA = 2,100 ± 263 kcal vs. EA = 2,200 ± 261 kcal vs. EA-OC = 2,000 ± 166 kcal). The average daily expenditure was different among the groups (AA = 2,400 ± 173 kcal vs. EA = 2,200 ± 115 kcal vs. EA-OC = 2,600 ± 302 kcal; \( P = 0.04 \)). Tukey’s post hoc test indicate that the EA-OC had a higher daily expenditure compared with EA (\( P = 0.035 \)).

**Vagal Tone**

There were no differences in vagal tone among the groups, as assessed by R-R variation during paced breathing, deep breathing, and the Valsalva ratio. During paced breathing, high-frequency power was not different (AA = 14,437 ± 4,600 ms\(^2\), EA = 11,500 ± 2,700 ms\(^2\), EA-OC = 21,000 ± 4,800 ms\(^2\)). Similarly, the MCR determined from R-R variation during deep breathing did not differ among the three groups (AA = 53 ± 8, EA = 67 ± 7, EA-OC = 59 ± 14). The mean MCR data for each group in the present study fell within the normative data ranges as determined by other investigators (12). There were no differences among the groups for the Valsalva ratio (AA = 1.75 ± 0.08, EA = 1.78 ± 0.06, EA-OC = 1.79 ± 0.11).

**Cardiovascular BRS**

The three indexes of BRS are shown in Fig. 3. The spontaneous index of BRS was not different among the groups (AA = 30 ± 6, EA = 24 ± 3, EA-OC = 29 ± 5 ms/mmHg). Cardiovascular BRS calculated from phase II of the Valsalva maneuver was not different among the groups (AA = 8 ± 2, EA = 7 ± 1, EA-OC = 8 ± 1 ms/mmHg). There was a difference in cardiovascular BRS derived from phase IV of the Valsalva maneuver among the groups (AA = 21 ± 3, EA = 15 ± 1, EA-OC = 26 ± 6 ms/mmHg; ANOVA \( P = 0.04 \)). Tukey’s post hoc test indicated that EA-OC had a higher cardiovascular BRS compared with EA (\( P = 0.04 \)). The three indexes of cardiovascular BRS did not correlate with baseline 17β-estradiol or progesterone concentration (data not shown).

**Cardiovascular Responses to Orthostatic Stress**

Baseline HR (AA = 49 ± 2, EA = 55 ± 2, EA-OC = 53 ± 3 beats/min) and MAP (AA = 74 ± 3, EA = 72 ± 3, EA-OC = 75 ± 5 mmHg). The three indexes of cardiovascular BRS were averaged for each group. There were no differences among the groups with regards to height, weight, \( \dot{V}O_2 \text{max} \), percent body fat, and bone mineral density (BMD). AA were slightly younger than EA-OC. All baseline blood work was within clinically acceptable normal limits for all groups, except one AA subject had a lower than normal hemoglobin of 10.5 g/dl. There were no differences in hematocrit, hemoglobin, red blood cell count, glucose, sodium, potassium, chloride, creatinine, calcium, blood urea nitrogen, aspartate transaminase, or alanine transaminase among the groups.

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Group</th>
<th>AA (n = 13)</th>
<th>EA (n = 13)</th>
<th>EA-OC (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>19 ± 1*</td>
<td>21 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166 ± 2</td>
<td>165 ± 2</td>
<td>164 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>57 ± 2</td>
<td>58 ± 2</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>( V\dot{O}_2 \text{max}, \text{ml.kg}^{-1}.\text{min}^{-1} )</td>
<td>49 ± 3</td>
<td>51 ± 2</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>22 ± 2</td>
<td>22 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>BMD, g/cm(^2)</td>
<td>1.14 ± 0.02</td>
<td>1.19 ± 0.02</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>( r )-score</td>
<td>0.41 ± 0.2</td>
<td>1.00 ± 0.3</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. AA, amenorrheic athletes; EA, eumenorrheic athletes; EA-OC, EA on oral contraceptives; \( V\dot{O}_2 \text{max} \), maximal oxygen uptake; BMD, bone mineral density. *\( P < 0.05 \) AA vs. EA-OC.

**Table 2. Reproductive hormones**

<table>
<thead>
<tr>
<th>Group</th>
<th>AA (n = 12)</th>
<th>EA (n = 10)</th>
<th>EA-OC (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol (ng/l)</td>
<td>40 ± 6</td>
<td>46 ± 8</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>Progesterone, µl</td>
<td>0.72 ± 0.09*</td>
<td>0.69 ± 0.05†</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>Total T(_2), ng/ml</td>
<td>0.04 ± 0.06*</td>
<td>1.02 ± 0.04†</td>
<td>1.44 ± 0.07</td>
</tr>
<tr>
<td>TSH, mUI</td>
<td>1.91 ± 0.42</td>
<td>2.07 ± 0.45</td>
<td>1.96 ± 0.55</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>83.2 ± 1</td>
<td>81.9 ± 1.6</td>
<td>78 ± 1.6</td>
</tr>
<tr>
<td>Sodium, mm/l</td>
<td>141 ± 0.3</td>
<td>141.1 ± 0.6</td>
<td>139.9 ± 0.6</td>
</tr>
<tr>
<td>Potassium, mm/l</td>
<td>4.3 ± 0.07</td>
<td>4.2 ± 0.08</td>
<td>4.3 ± 0.09</td>
</tr>
<tr>
<td>Chloride, mm/l</td>
<td>103 ± 0.3</td>
<td>104.5 ± 0.5</td>
<td>105.5 ± 0.5</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>13.4 ± 1</td>
<td>13.2 ± 0.8</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.92 ± 0.03</td>
<td>0.88 ± 0.02</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>Calcium, mg/dl</td>
<td>9.59 ± 0.08</td>
<td>9.28 ± 0.12</td>
<td>9.24 ± 0.15</td>
</tr>
<tr>
<td>AST, units/l</td>
<td>29.9 ± 3</td>
<td>30.5 ± 2</td>
<td>33.5 ± 2</td>
</tr>
<tr>
<td>ALT, units/l</td>
<td>33.7 ± 3</td>
<td>27.4 ± 2.4</td>
<td>27.2 ± 2</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>4.9 ± 0.11*</td>
<td>4.8 ± 0.09</td>
<td>4.4 ± 0.11</td>
</tr>
<tr>
<td>RBC, cells pl</td>
<td>4.6 ± 0.12</td>
<td>4.7 ± 0.06</td>
<td>4.6 ± 0.09</td>
</tr>
<tr>
<td>Hgb, g/dl</td>
<td>13.6 ± 0.5</td>
<td>13.6 ± 0.4</td>
<td>13.9 ± 0.3</td>
</tr>
<tr>
<td>Hct, %</td>
<td>41.4 ± 1</td>
<td>40.7 ± 1</td>
<td>41.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. TSH, thyroid-stimulating hormone; BUN, blood urea nitrogen; AST, aspartate transaminase; ACT, alanine transaminase; RBC, red blood cells; Hgb, hemoglobin; Hct, hematocrit. *\( P < 0.05 \) AA vs. EA-OC. †\( P < 0.05 \) EA vs. EA-OC.
EA-OC = 82 ± 5 mmHg) were not different among groups. HR responses during the 20-min tilt are shown in Fig. 4A. HR increased at each time point during the tilt compared with baseline (ANOVA, time \( P < 0.01 \)). There were no differences in HR during the tilt among the groups. MAP responses to orthostatic stress are shown in Fig. 4B. MAP was well maintained throughout the tilt. There were no differences in MAP among the groups. Limb vascular resistance increased during the tilt in all groups (\( P < 0.05 \)); however, there were no group differences. To account for minor differences in baseline limb vascular resistance, we additionally compared the absolute change from baseline to the end of the tilt, and there were also no differences (\( \text{AA} = 9 \pm 5, \text{EA} = 12 \pm 5, \text{EA-OC} = 22 \pm 8 \)). During the tilt, two EA subjects became syncopal and therefore were not used in the analysis. No AA or EA-OC subjects experienced symptoms of syncope during the tilt test.

**DISCUSSION**

In the present study we examined autonomic function in three groups of female athletes: AA, EA, and EA-OC. Multiple indexes were used to examine autonomic function and included measurements of vagal tone, cardiovagal BRS, and cardiovascular responses to orthostatic stress. Our hypothesis was that AA would have attenuated autonomic function, shown by a lower cardiovagal BRS and vagal tone. Experimental animal studies have demonstrated that both acute and chronic exposure to estradiol can improve BRS (9, 29). In addition, recent studies have shown that flow-mediated dilation (a marker of cardiovascular health) is attenuated in AA; this is reversed when oral contraceptives are administered to this group (28, 38). A chronic state of hypoestrogenism may have long-term cardiovascular effects in these women, and it was the intent of this study to determine whether AA have attenuated autonomic cardiovascular control. However, our results show that AA do not display signs of impaired autonomic function compared with EA and EA-OC during the follicular phase of the menstrual cycle, indicating that women that are amenorrheic for a short period of time do not have attenuated autonomic function. However, we cannot extend these conclusions to comparisons between AA and EA or EA-OC during the luteal phase of the menstrual cycle.

To the best of our knowledge, autonomic function in AA has not been studied. However, the sympathetic and parasympathetic branches of the autonomic nervous system have been examined in eumenorrheic women and women using oral contraceptives. These studies measured BRS at two or more time points across the menstrual cycle to determine the impact of fluctuating reproductive hormones (14, 20, 21, 34). Results in eumenorrheic women are conflicting with respect to cardiovagal BRS (14, 20). When comparing the early follicular and midluteal phases of the menstrual cycle, Minson et al. (20) did not report any differences in cardiovagal BRS, although sympathetic BRS was higher in the luteal phase. Conversely, Hirshoren et al. (14) reported an increase in cardiovagal BRS during the luteal phase. Tanaka et al. (34) found an increase in BRS in the preovulatory phase of the menstrual cycle. However, Cooke et al. (5) did not find any differences in BRS across four time points of the menstrual cycle. Thus, in eu-
Amenorrheic women with cyclic changes in reproductive hormones, it is unclear whether the acute monthly changes in estradiol and progesterone concentrations impact BRS. A separate study by Minson et al. (21) on women taking oral contraceptives reported that cardiovagal BRS was higher during the placebo phase of oral contraceptive use. Endogenous reproductive hormones are suppressed during oral contraceptive use until the placebo phase. During the placebo phase, endogenous production remains suppressed until the concentration of exogenous hormones has withdrawn (7, 31, 36).

Therefore, if the endogenous concentrations are suppressed, it is likely that exogenous concentrations are still present. Thus chronic exposure to increased levels of reproductive hormones from exogenous estradiol and progesterone may increase BRS; alterations in BRS may be different between women on and off oral contraceptives. Collectively, these studies demonstrate that autonomic function is affected by reproductive hormones. Perhaps the cyclicity of (or surges in) reproductive hormones, rather than the levels of hormones themselves, are important in regulating autonomic function.

In the present study with our subject population, three separate measurements of BRS were performed, all showing that autonomic function was not lower in amenorrheic athletes. Eumenorrheic athletes using oral contraceptives in our study had a higher cardiovagal BRS analyzed from phase IV of the Valsalva compared with eumenorrheic controls. Because the endogenous surge had not occurred (based on the suppressed hormonal values), it is likely that these subjects were still exposed to exogenous hormone levels during the time of testing. Therefore, it is interesting that this group had a higher cardiovagal BRS and most likely was still experiencing high concentrations of reproductive hormones during the placebo phase. However, the purpose of this study was to determine whether AA had decreases in autonomic function because reproductive hormones are chronically suppressed. In comparison, eumenorrheic women have normal cyclic changes in reproductive hormones, and oral contraceptive users are exposed to high concentrations of hormones. We standardized the testing with respect to the menstrual cycle to avoid the confounding effects of acute alterations in circulating estradiol to ensure that the AA and EA groups would be as similar as possible. This approach is consistent with the study hypothesis, because it was not our intent to tease out the effects of estradiol but rather to determine whether the chronic suppression of reproductive hormones leads to alterations in autonomic function. Our intent was to determine whether chronic (i.e., 3 mo or longer) low estradiol affected BRS. For this reason, we did not examine autonomic function during multiple time points across the menstrual cycle to determine the acute effects of fluctuations in reproductive hormones on BRS. It was our purpose to determine whether the lack of chronic exposure to reproductive hormones were associated with changes in autonomic function.

Our main measurement of cardiovagal BRS was the Valsalva maneuver, which correlates to vasoactive measures of BRS like the Oxford technique (1). In our study, the BRS values were consistently lower when derived from the pressure declines compared with the pressure increases; this is consistent with reported measurements using the vasoactive drug technique. One study measured multiple indexes of BRS, including vasoactive drugs, the Valsalva maneuver, and a spontaneous index of BRS, across three phases of the menstrual cycle (34). A correlation between the Valsalva and the reflex response to a vasoactive drug-induced pressure increase was reported, supporting the use of the Valsalva maneuver to assess BRS (34). The spontaneous index of BRS has been proposed as a noninvasive way to estimate baroreflex function, demonstrated by a correlation between spontaneous BRS and the reflex responses to vasoactive drugs (25, 34). However, recent data suggest that a spontaneous index of BRS may be related more to vagal tone than baroreflex function (17). In our data set, the spontaneous index of BRS was highly correlated to the high-frequency power (Pearson’s correlation coefficient = 0.871, P < 0.01) and also correlated to the MCR (Pearson’s correlation coefficient = 0.475, P = 0.012) but not Valsalva-derived BRS. In summary, the methodologies employed to assess BRS in the present study are frequently used in the human literature, and importantly, these indexes do not show lower BRS values in the AA group.

Tilt-table testing is commonly used to assess the presence of orthostatic intolerance. Impaired cardiovagal BRS and/or impaired sympathetic activity (e.g., sympathetic vasoconstriction) may cause symptoms of orthostatic intolerance and orthostatic hypotension. In the present study, there were no differences in the blood pressure and HR responses to orthostatic stress. There was an initial decline in MAP in the EA and EA-OC groups with the tilt, but the mean data from the AA group did not show that decline. Pressure was well maintained throughout the tilt in all groups. Contrary to our initial hypothesis, no AA experienced signs of orthostatic intolerance. However, two EA subjects experienced syncope during the tilt. These two subjects were not outliers in any other variables measured. Sex differences have previously been reported in cardiovascular responses to orthostatic stress, and blood pressure declines were greater in women compared with men during lower body negative pressure (3).

Amenorrheic women have been reported to have lower levels of estradiol, along with TSH hormone and T3 (18, 19). In the present study, we did not find AA to have lower estradiol or progesterone, which was also found in a recent study (38). One possible reason there were no differences in estradiol may be from testing the control group in the early follicular phase of the menstrual cycle, where both estradiol and progesterone are low. Estradiol was reported to be lower in two studies by Loucks et al. (18, 19) when AA were compared with sedentary eumenorrheic women, but estradiol concentration was not lower compared with athletic eumenorrheic women. However, Harber et al. (13) reported estradiol to be lower in AA compared with both trained and untrained eumenorrheic women.

One reason amenorrhea may occur is from a metabolic disturbance. Metabolic markers such as T3 and TSH have been reported to be lower in amenorrheic women. Total T3 and thyroxine (T4) were reported to be lower in athletic amenorrheic women compared with sedentary eumenorrheic women, but not athletic eumenorrheic women (18). A separate study reported T3 and T4 to be lower in athletic amenorrheic women compared with athletic eumenorrheic women and eumenorrheic sedentary controls (13). In the present study, AA had lower levels of total T3 compared with EA-OC. Interestingly, EA also had a lower total T3 compared with EA-OC, although...
no groups were different in TSH or in their caloric intake. In
the present data set, we are unable to distinguish among the
groups based on the metabolic markers measured in this study.

Although the mean t-score and BMD tended to be lower in
the AA group, there were no differences among the groups in
the present study. However, in a similar study with 14 amen-
orrhoeic subjects, no differences were reported in vertebral
BMD compared with controls (38). As part of the initial
screening of subjects, a standard blood panel was performed to
ensure there were no imbalances in electrolytes and in renal,
and hepatic enzymes. This was to ensure that all subjects were
similar and that no other underlying disorders were impacting cardiovascular health.

There are several limitations that should be mentioned. First, the Valsalva maneuver may not be sensitive enough to detect
small changes in BRS, and because of the short duration of the
maneuver, there are limited data points available to calculate a
slope. For example, the sigmoid nature of the curve is not
consistently apparent when using Valsalva-induced blood pres-
sure changes. In calculating the slope, the linear portion of the
curve is used. In addition, data collected during the Valsalva
maneuver do not purely represent autonomic function. Along
with autonomic effects, there are hemodynamic and physical
effects on thoracic volume. However, Monahan et al. (22)
was able to show a difference in BRS using the Valsalva maneuver
between sedentary and exercising male subjects. Cardiovagal
BRS, measured by the Valsalva, increased after a 4-wk aerobic
training program in healthy male subjects (6). The studies by
both Monahan et al. (22) and Cooke et al. (6) reinforce the use
of the Valsalva maneuver as a tool for measuring BRS; therefore,
the Valsalva maneuver can be used as a measure of
BRS in an athletic population. Second, hydration was not
controlled for during the screening or the testing session. Third,
subjects in the oral contraceptive group were using different
oral contraceptive regimens; concentration and phasic use of
oral contraception may impact autonomic function differently.
However, no correlations were found between estradiol hor-
mone concentrations and BRS, and the assays show endoge-
 nous production was suppressed in this group. Fourth, in the
present experimental design, we do not test BRS during the
luteal phase of the menstrual cycle when reproductive hor-
mones are higher. As stated, the purpose of the study was not
to compare the acute effect of monthly cyclic changes in
reproductive hormones on cardiovascular function but to de-
termin whether chronic lack of exposure to reproductive hormones affected autonomic function. For this reason, we
included the group on oral contraceptives, which represent a
group of women who are exposed to high levels of exogenous
hormones. Despite these limitations, several indexes show that
AA do not display signs of impaired autonomic function.

In conclusion, autonomic function was not lower in AA
athletes compared with two groups of EA. To our knowledge,
this is the first study to examine indexes of autonomic function
in this population. Multiple indexes were used to test auto-
nomic cardiovascular control in female athletes, and collec-
tively they showed that AA do not display signs of impaired
autonomic function and orthostatic responses compared with
EA or EA-OC during the follicular phase of the menstrual
cycle. However, we cannot dismiss the possibility that chron-
ically low levels of estrogen and progesterone may impair

autonomic function in AA compared with EA or EA-OC
during the luteal phase of the menstrual cycle.

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