Discordant Orthostatic Reflex Renin–Angiotensin and Sympathoneural Responses in Premenopausal Exercising-Hypoestrogenic Women

Emma O’Donnell, Jack M. Goodman, Susanna Mak, Hisayoshi Murai, Beverley L. Morris, John S. Floras, Paula J. Harvey

Abstract—Our prior observations in normotensive postmenopausal women stimulated the hypotheses that compared with eumenorrheic women, active hypoestrogenic premenopausal women with functional hypothalamic amenorrhea would demonstrate attenuated reflex renin–angiotensin–aldosterone system responses to an orthostatic challenge, whereas to defend blood pressure reflex increases in muscle, sympathetic nerve activity would be augmented. To test these hypotheses, we assessed, in recreationally active women, 12 with amenorrhea (ExFHA; aged 25±1 years; body mass index 20.7±0.7 kg/m²; mean±SEM) and 17 with eumenorrhea (ExOv; 24±1 years; 20.9±0.5 kg/m²), blood pressure, heart rate, plasma renin, angiotensin II, aldosterone, and muscle sympathetic nerve activity at supine rest and during graded lower body negative pressure (−10, −20, and −40 mmHg). At baseline, heart rate and systolic blood pressure were lower (P<0.05) in ExFHA (47±2 beats/min and 94±2 mmHg) compared with ExOv (56±2 beats/min and 105±2 mmHg), but muscle sympathetic nerve activity and renin–angiotensin–aldosterone system constituents were similar (P>0.05). In response to graded lower body negative pressure, heart rate increased (P<0.05) and systolic blood pressure decreased (P<0.05) in both groups, but these remained consistently lower in ExFHA (P<0.05). Lower body negative pressure elicited increases (P<0.05) in renin, angiotensin II, and aldosterone in ExOv, but not in ExFHA (P>0.05). Muscle sympathetic nerve activity burst incidence increased reflexively in both groups, but more so in ExFHA (P<0.05). Otherwise, healthy hypoestrogenic ExFHA women demonstrate low blood pressure and disruption of the normal circulatory response to an orthostatic challenge: plasma renin, angiotensin II, and aldosterone fail to increase and blood pressure is defended by an augmented sympathetic vasoconstrictor response. (Hypertension. 2015;65:1089-1095. DOI: 10.1161/HYPERTENSIONAHA.114.04976.) • Online Data Supplement

Key Words: blood pressure • estrogens • exercise • renin-angiotensin system • sympathetic nervous system

Functional hypothalamic amenorrhea (FHA) is a reversible cause of ovarian suppression in premenopausal women. Its prevalence is markedly higher in recreationally and competitively exercising women and women with physically demanding jobs (≈1% to 44%) than in sedentary women (≈2% to 5%). Hypothalamic-pituitary-ovarian suppression in physically active women with FHA (ExFHA) has been related causally to energy deficiency (ie, caloric deficit) in association with high energy expenditure and insufficient energy intake (ie, low caloric intake). Circulating estradiol concentrations in women with ExFHA are chronically low and resemble those observed in postmenopausal women and in men. In postmenopausal women, estrogen deficiency is associated with increases in blood pressure (BP) and a marked acceleration in the development and progression of atherosclerosis. Cardiovascular consequences of premenopausal hypoestrogenemia in ExFHA women are not yet known, but disruption of the menstrual cycle during the reproductive years is also associated with the premature development and progression of coronary artery disease. However, in contrast to postmenopausal women, ExFHA women have lower resting systolic blood pressure (SBP) and heart rate (HR) than age- and fitness-matched estrogen-replete physically active women. The presence of low arterial BP, despite hypoestrogenemia, suggests that estrogen deficiency may have different consequences for BP regulation in premenopausal and postmenopausal women.

The sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS) are cornerstones of BP regulation. Estrogen modulates both. In estrogen-deficient
postmenopausal women, efferent skeletal muscle sympathetic nerve activity (MSNA) is elevated and renin mass, plasma renin activity, angiotensin II, and aldosterone fail to increase reflexively in response to simulated orthostatic stress, whereas exogenous estrogen increases circulating levels of angiotensinogen, angiotensin I, renin, and angiotensin II and decreases MSNA.

The purpose of this study was to investigate, for the first time, the consequences of hypoestrogenemia in premenopausal women for BP regulation. We compared, in ExFHA women and estrogen-replete eumenorrheic women, MSNA burst incidence and plasma renin, angiotensin II and aldosterone concentrations both at rest and during an orthostatic hypotensive challenge (graded lower body negative pressure, LBNP) applied to stimulate reflexively sympathetic outflow and the RAAS. We anticipated, from the literature, that women with ExFHA would have lower BP than eumenorrheic women both at rest and during LBNP. Based on our prior findings in postmenopausal women, we hypothesized that their reflex RAAS response to this orthostatic challenge would be attenuated, whereas to defend BP their reflex increase in MSNA, burst incidence would be augmented.

Methods

Subjects

Volunteers were recruited by posters targeting currently physically active premenopausal women. Screening procedures included general questionnaires on exercise habit, eating behavior, menstrual cycle, and medical health history. Eligibility criteria for the study included (1) age 18 to 35 years; (2) good health determined by absence of chronic illnesses, including diabetes mellitus, hyperprolactinemia, poly-cystic ovarian syndrome, and thyroid disease; (3) stable menstrual status over the preceding 3 months defined as (i) menstrual cycles between 25 and 35 days for menstruating women or (ii) complete absence of menses for at least 90 consecutive days in amenorrheic women; (4) taking no medications; (5) nonsmoker; (6) not currently dieting and weight stable for the preceding 3 months; (7) absence of hormonal therapy for at least 6 months; (8) no history of structured exercise for >6 months and a peak aerobic capacity of ≥40 mL/kg/min; (9) not currently participating in any therapeutic or clinical research involving unpredictable menstrual cycles of variable length (ie, oligomenorrhea) or (i0) current clinical diagnosis of eating disorders; (9) participating in regular questionnaires on exercise habit, eating behavior, menstrual cycles between 25 and 35 days for menstruating women and estrogen-replete eumenorrheic women, MSNA burst incidence and plasma renin, angiotensin II, and aldosterone fail to increase reflexively in response to simulated orthostatic stress, whereas exogenous estrogen increases circulating levels of angiotensinogen, angiotensin I, renin, and angiotensin II and decreases MSNA.14

Study Groups

Two groups of physically active women were established: eumenorrheic ovulatory women (ExOv; n=17), and FHA women (ExFHA; n=12), defined as cessation of menses for ≥90 days.7 Women reporting unpredictable menstrual cycles of variable length (ie, oligomenorrhea) were excluded. Exercising status was defined as ≥2 hours/week of structured exercise for >6 months and a peak aerobic capacity of ≥40 mL/kg/min.10 In eumenorrheic women, ovulatory status was confirmed in the menstrual cycle before testing by using a commercial urinary ovulation hormone kit (Clearblue Easy; Unipath Diagnostics, Waltham, MA).

Subject Preparation

All measures were obtained during the early follicular phase (low estrogen and progesterone; days 2–6) of the menstrual cycle in eumenorrheic subjects and on a random day (low estrogen and progesterone) for amenorrheic women. Thus, we were able to compare the cardiovascular effects of cyclically low estrogen versus chronically low estrogen levels. All tests occurred in the morning between 0930 and 1030 hours in a quiet ambient temperature room (22–24°C). Volunteers had fasted at least 2 hours and abstained from alcohol 12 hours, and caffeine and exercise for 24 hours, before testing.

Anthropometric Measures and Body Composition

Total body mass and height were determined using a physician’s balance scale (Detecto, Webb City, MO). Body composition was determined using dual-energy x-ray absorptiometry (DXA;Prodigy, General Electric Lunar Corporation, Madison, WI). Central fat mass was determined from the fat mass (kg) measured in the trunk, and peripheral fat mass was determined as the sum of fat mass (kg) measured in the arms and legs.

Peak Aerobic Capacity

On a separate study day, peak aerobic capacity (VO2 peak) was measured using a metabolic cart during a progressive treadmill test to exhaustion (Moxus Modular VO2 System; Applied Electrochemistry Inc., Pittsburgh, PA).

Blood Sampling

Blood sampling methods are described in detail in the online-only Data Supplement.

Blood Pressure and Heart Rate

Systolic (SBP), diastolic BP (DBP), mean arterial BP (MAP), and HR were recorded in the supine position from the left upper-arm using an automated device (Dinamap Pro 100, Critikon). Continuous recordings of HR and BP were also acquired using lead II of an ECG and a photoplethysmographic device on the index finger (Portapres Model-2; Finapres Medical Systems BV), respectively. Detailed BP and HR data acquisition methods are described in the Methods section in the online-only Data Supplement. Brachial BP and HR were assessed at 1 minute intervals at baseline (3 consecutive stable measures) and every minute throughout each stage of LBNP. The recordings acquired during the last 4 minutes of each LBNP stage were averaged to acquire a mean value for each LBNP stage.

Muscle Sympathetic Nerve Activity

Multi-unit recordings of postganglionic MSNA were obtained in the supine position with a unipolar tungsten electrode inserted selectively

<table>
<thead>
<tr>
<th>Measure</th>
<th>ExOv (n=17)</th>
<th>ExFHA (n=12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>24±1</td>
<td>25±1</td>
<td>0.308</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166±2</td>
<td>168±1</td>
<td>0.316</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>57.5±1.9</td>
<td>58.4±2.1</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.9±0.5</td>
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</tr>
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<td>Body fat, %</td>
<td>23.5±1.8</td>
<td>21.8±2.1</td>
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</tr>
<tr>
<td>Lean mass, kg</td>
<td>41.9±1.6</td>
<td>44.2±1.7</td>
<td>0.377</td>
</tr>
<tr>
<td>VO2 peak, mL/kg/min</td>
<td>47.1±1.3</td>
<td>46.3±1.9</td>
<td>0.666</td>
</tr>
<tr>
<td>Estradiol, pmol/L</td>
<td>154±24</td>
<td>87±13</td>
<td>0.045</td>
</tr>
<tr>
<td>Progesterone, nmol/L</td>
<td>1.6±0.2</td>
<td>1.4±0.2</td>
<td>0.634</td>
</tr>
<tr>
<td>Free T3, pmol/L</td>
<td>4.2±0.2</td>
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<td>0.017</td>
</tr>
<tr>
<td>Testosterone, nmol/L</td>
<td>2.1±0.1</td>
<td>2.4±0.3</td>
<td>0.246</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>51±3</td>
<td>51±4</td>
<td>0.982</td>
</tr>
<tr>
<td>FAI, au</td>
<td>4.6±0.3</td>
<td>4.8±0.7</td>
<td>0.762</td>
</tr>
</tbody>
</table>

Values mean±SEM. BMI indicates body mass index; FAI, free androgen index; SHBG, sex hormone binding globulin; T3, triiodothyronine; and VO2 peak, peak oxygen uptake.

*Range 150–3132 days.
into a muscle-nerve fascicle of the right fibular (peroneal) nerve using previously described techniques. Detailed MSNA methods are available in the online-only Data Supplement. Because the purpose of this component of the protocol was to compare the reflex effects of a stimulus (unloading of mechanoreceptor nerve afferents by LBNP) on central sympathetic outflow between 2 groups known to differ with respect to cardiac frequency, the principal outcome variable was the HR-independent measure, MSNA burst incidence (bursts/100 cardiac cycles), which is derived from burst frequency (bursts/min) and HR.

LBNP
With subjects supine, the lower body was encased in a custom-built chamber sealed at the level of the iliac crest and attached to a vacuum source. A trap door permitted lower leg access for microelectrode placement. HR, BP, respiratory excursions, and MSNA were recorded continuously at baseline and during each stage of LBNP. MSNA was recorded in 8-minute stages at baseline and during sequential graded application of LBNP at −10, −20, and −40 mm Hg. Each LBNP stage was followed by 5-minutes of recovery (ie, no LBNP). LBNP was terminated if SBP fell <80 mm Hg, pallor was observed, or subjective feelings of nausea, dizziness, or light-headedness were reported.

Statistics
All data sets were tested for non-normality, homogeneity of variance, and outliers. All data sets were normally distributed and no outliers were detected. Group differences at baseline were detected using 1-way analysis of variance. Within- and between-group analyses of responses to LBNP, including change (Δ) in measures compared with baseline, were determined using repeated measures analysis of variance. When assumptions of sphericity were violated, the Greenhouse–Geisser correction was used. Using separate data for each group, Pearson’s correlational analyses were used to determine significant linear independent associations between BP and neurohumoral variables of interest. Data were analyzed using packaged software (SPSS version 20; SPSS Inc., Chicago, IL). All data are presented as the mean±SEM. A significance level of P<0.05 was used to detect the differences for statistical procedures.

Figure. Graphs showing the effects of lower body negative pressure in exercising eumenorrheic women (ExOv; closed circles) and amenorrheic women (ExFHA; open circles) on cardiovascular measures, including hemodynamic measures of systolic blood pressure (SBP; A), mean arterial blood pressure (MAP; B), heart rate (HR; C); serum measures of renin (D), angiotensin II (Ang II; E), and aldosterone (F); and muscle sympathetic nerve activity (MSNA), assessed as burst frequency (bursts/min; G), burst incidence (bursts/100 heart beats; H), and change in burst incidence from baseline (I). *P<0.05 between-groups for the given condition. Repeated measures analysis of variance for effects of lower body negative pressure (LBNP), GROUP, and interactions between these factors (LBNP×GROUP) are reported separately on each graph. Values are mean±SEM.
### Results

#### Subject Characteristics

The study groups did not differ \((P>0.05)\) in age, height, weight, body mass index, body composition, body fat distribution, cardio-respiratory fitness level, free androgen index, or serum measures of testosterone, progesterone, and follicle stimulating hormone (Table 1). In contrast, serum estrogen, free T3, and luteinizing hormone concentrations were significantly lower \((P<0.05)\) in ExFHA women.

#### Baseline Values

Resting BP and HR values for the 2 study groups \((n=29)\) differed between the groups. Specifically, SBP, MAP, and HR were lower \((P<0.05)\) in ExFHA women compared with ExOv women (Figure). DBP did not differ between the groups \((P=0.25)\). Acceptable microelectrode recordings of MSNA were obtained in 22 of the 29 women \((ExOv, n=12;\ ExFHA, n=10)\). Despite lower resting HR in ExFHA women, MSNA burst incidence \((\text{bursts/100 heart beats})\) did not differ \((P>0.05)\) between groups (Table 2). Differences in baseline MSNA burst frequency \((\text{bursts/minute})\) were also not detected \((P>0.05)\). Blood samples for RAAS components were successfully collected in 20 subjects \((ExOv, n=11;\ ExFHA, n=9)\). Of the 9 subjects that were not tested for RAAS, canulization was unsuccessful in 7 women and was refused by 2 women. RAAS components, including the aldosterone/renin ratio, did not statistically differ \((P>0.05)\) between the groups (Table 2).

#### Responses to LBNP

LBNP elicited similar \((P>0.05)\) within-group decreases in SBP, MAP, and DBP \((P<0.001\ for\ all\ measures)\) and increases \((P<0.001)\) in HR (Figure). Significant differences in between-group responses to graded LBNP were observed for SBP \((P<0.01)\) and HR \((P<0.01)\), with ExFHA demonstrating consistently lower values than ExOv women. Although between-group DBP values during LBNP did not differ \((P=0.45)\), MAP trended \((P=0.06)\) toward lower values in ExFHA. ∆HR, ∆SBP, ∆DBP, and ∆MAP values during LBNP did not differ \((P>0.05;\ all\ measures)\) between the groups. LBNP did not elicit presyncopal symptoms in any subject.

Within groups, LBNP caused MSNA burst frequency \((P<0.001)\ and\ burst\ incidence\ \((P<0.001)\ to\ increase\ similarly\ \((P>0.05)\ in\ both\ groups\ (Figure).\ In\ contrast,\ between\ groups,\ MSNA\ adjusted\ for\ HR\ (burst\ incidence;\ \(P=0.04)\, but\ not\ burst\ frequency\ \((P>0.05)\, was\ significantly\ higher\ in\ ExFHA\ versus\ ExOv\ women\ at\ −20\ and\ −40\ mm\ Hg.\ ∆MSNA\ burst\ incidence,\ but\ not\ frequency,\ also\ demonstrated\ significantly\ greater\ \((P<0.05)\ values\ in\ ExFHA\ versus\ ExOv\ women\ at\ −20\ and\ −40\ mm\ Hg\ (Figure).

Within groups, LBNP elicited significant increases in renin \((P<0.01)\) and Ang II \((P=0.01)\, but\ not\ aldosterone\ \((P>0.05)\;\ Figure).\ However,\ significant\ \((P<0.05)\ between-group

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**Table 2. Neurohumoral Measures at Baseline and During Graded LBNP (mm Hg)**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Ba</th>
<th>−10</th>
<th>−20</th>
<th>−40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin, pmol/L*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExOv</td>
<td>8.0±2.0</td>
<td>7.4±1.8</td>
<td>8.7±2.0</td>
<td>12.5±2.5†</td>
</tr>
<tr>
<td>ExFHA</td>
<td>4.0±0.8</td>
<td>4.2±0.8</td>
<td>4.3±0.7‡</td>
<td>4.8±0.9‡</td>
</tr>
<tr>
<td>Ang II, pmol/L*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExOv</td>
<td>3.9±0.8</td>
<td>4.1±0.9</td>
<td>5.3±1.4†</td>
<td>8.6±1.9†</td>
</tr>
<tr>
<td>ExFHA</td>
<td>2.3±0.3</td>
<td>2.6±0.4</td>
<td>2.1±0.3‡</td>
<td>2.0±0.4‡</td>
</tr>
<tr>
<td>Aldo, pmol/L*</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ExOv</td>
<td>88±16</td>
<td>74±8</td>
<td>77±9</td>
<td>134±30</td>
</tr>
<tr>
<td>ExFHA</td>
<td>132±32</td>
<td>117±32</td>
<td>104±25</td>
<td>96±27</td>
</tr>
<tr>
<td>Aldo/Renin, pmol/L*</td>
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<td></td>
<td></td>
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<tr>
<td>ExOv</td>
<td>15±3</td>
<td>17±4</td>
<td>19±10</td>
<td>36±25</td>
</tr>
<tr>
<td>ExFHA</td>
<td>34±10</td>
<td>29±10</td>
<td>25±7</td>
<td>21±6</td>
</tr>
<tr>
<td>MSNA (frequency)§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExOv</td>
<td>8±2</td>
<td>10±2</td>
<td>13±2†</td>
<td>22±3†</td>
</tr>
<tr>
<td>ExFHA</td>
<td>10±2</td>
<td>13±3</td>
<td>18±2†</td>
<td>27±2†</td>
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<tr>
<td>MSNA (incidence)§</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ExOv</td>
<td>15±3</td>
<td>18±4</td>
<td>22±4†</td>
<td>33±4†</td>
</tr>
<tr>
<td>ExFHA</td>
<td>22±5</td>
<td>28±6</td>
<td>37±4‡</td>
<td>45±4‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Aldo indicates aldosterone; ald/o, aldosterone/renin ratio; Ang II, angiotensin II; Ba, baseline; frequency, burst frequency measuring number of bursts per minute; incidence, burst incidence measuring number of bursts per 100 heart beats; LBNP, lower body negative pressure; and MSNA, muscle sympathetic nerve activity.

*ExOv, n=11; ExFHA, n=10.
†Significantly different from baseline within-groups, \(P<0.05\).
‡Significantly different between-groups for given LBNP, \(P<0.05\).
§ExOv, n=12; ExFHA, n=10.
interactions in response to LBNP were observed for each RAAS component. Notably, in contrast to ExOv women, ExFHA women demonstrated no activation of either renin or Ang II during LBNP (Figure). Although not significant ($P>0.05$), aldosterone appeared to be decreased in response to LBNP in ExFHA, yet increased in ExOv women. The aldosterone/renin ratio (Table 2) did not differ ($P>0.05$) between the groups. Within- and between-group analyses using $\Delta$ measures of each RAAS component did not alter the findings.

**Correlates**

Using separate data for each group, no significant correlates ($P>0.05$) were detected between MSNA, BP, and RAAS. All other circulating hormones were also not related ($P>0.05$) to these variables.

**Discussion**

This, to our knowledge, is the first study to report hemodynamic, effenter sympathetic nerve discharge, and plasma renin, angiotensin II, and aldosterone concentrations both at rest and in response to their acute reflex activation by simulated orthostatic stress in young estrogen-deficient physically active premenopausal women with FHA (ExFHA). This experiment yielded several novel findings. Compared with body mass index-, body composition-, age-, and fitness-matched eumenorrheic women, hypoestrogenic women with FHA demonstrated (i) similar tonic basal efferent sympathetic outflow to the lower limb and similar plasma renin, angiotensin II, and aldosterone concentrations, yet lower resting HR and SBP; (ii) no reflex activation of renin, angiotensin II, or aldosterone in response to LBNP; and (iii) consistently lower BP and HR during this orthostatic challenge, despite greater reflex increases in muscle sympathetic burst incidence. These findings are consistent with the concept that hypoestrogenic ExFHA women rely on augmented sympathoneural vasoconstrictor responsiveness to maintain BP when standing.

Compared with eumenorrheic women, the BP and HR of ExFHA women were significantly lower and sympathetic burst incidence tended to be higher. The absence of correlation between resting arterial BP and MSNA in either ExFHA or ExOv women is consistent with prior observations in men and in women <40 years old.$^{16,17}$ In both groups of women, graded LBNP elicited the anticipated reflex increase in MSNA burst incidence.$^{18}$ However, the finding that the magnitude of this response is greater in ExFHA than in ExOv women has not been previously reported. Although the precise central mechanism responsible for greater reflex sympatho-excitation in hypoestrogenic women remains to be determined, human and animal studies have established that both endogenous and exogenous estrogen lower resting sympathetic discharge.$^{14,19,20}$ Absence of estrogen-mediated sympatho-inhibition would be anticipated to increase MSNA burst incidence. By contrast, information concerning the effects of endogenous and exogenous estrogen on reflex MSNA responses to orthostatic stress is both limited and inconsistent. For example, head-up tilt has been reported to elicit both lower$^{21}$ and similar$^{22}$ MSNA responses in young women compared with men. Simulated orthostatic stress augments MSNA more during the mid-luteal phase (high estrogen and progesterone) than in the early follicular phase (low estrogen and progesterone) of the menstrual cycle.$^{23,24}$ Combined oral hormonal contraceptives in premenopausal women$^{25}$ and transdermal estrogen therapy in postmenopausal women$^{26}$ are reported not to influence the magnitude of MSNA increases elicited by orthostatic stress. The reason for such conflicting findings has yet to be resolved but difference in the mode of orthostatic challenge, the comparison of endogenous versus exogenous estrogen preparations, and confounding effects of progesterone$^{20}$ are plausible candidates.

It might be anticipated from their lower BP both at rest and during LBNP and from their augmented MSNA response that the plasma renin, angiotensin II, and aldosterone of ExFHA women would be significantly greater than those of their ExOv counterparts under both resting conditions and in response to this orthostatic challenge. However, the opposite was observed. Unlike ExOv women, in whom renin and angiotensin II increased reflexively as expected in response to lowering of systolic BP with graded LBNP,$^{12,27}$ LBNP had no effect on the plasma renin, angiotensin II, or aldosterone of ExFHA women. Although the causal mechanism responsible for the absent RAAS response in ExFHA women cannot be established with certainty, there is a large body of experimental evidence implicating estrogen deficiency. RAAS responses to graded LBNP are similarly absent in healthy normotensive postmenopausal women.$^{1}$ After 1 month of oral estradiol (2 mg daily), LBNP elicited reflex increases in plasma renin activity and angiotensin II corresponding to those observed in a comparator group of healthy premenopausal women studied under identical conditions.$^{12}$ Estrogen has been shown to upregulate angiotensinogen gene expression,$^{28}$ increase plasma angiotensin I and II concentrations, reduce angiotensin-converting enzyme activity, alter plasma renin concentrations,$^{12,13}$ and modulate local factors known to regulate renal renin secretion. For example, in the immediate vicinity of the juxtaglomerular cells, renal endothelial nitric oxide stimulates and endothelin inhibits renin secretion.$^{29}$ Thus, renal renin release may be diminished in estrogen-deficient states.

RAAS activity has also been associated positively with circulating thyroid hormone levels.$^{31}$ Thus, it is plausible that low triiodothyronine, as observed in ExFHA women in the current and in previous$^{10,11}$ studies, may also contribute to this low renin state. Considering the similarity of body mass index in our 2 groups and the clinical absence of edema, intravascular volume expansion is an unlikely RAAS-suppressive mechanism.

The authors acknowledge certain study limitations. Hydration status can influence MSNA responses to orthostatic stress,$^{22}$ whereas sodium depletion and protein overfeeding can increase RAAS activity.$^{52}$ Mild negative energy balance may contribute to hyperadiponectinemia, hypo leptinemia, and low triiodothyronine.$^{33,34}$ These factors were not controlled for in the current study. Because of our sample size, we may not have been able to detect associations between variables of interest that were in fact present. Unknown is whether tissue RAAS or novel RAAS components, such as ang-(1–7) and...
the MAS receptor, are altered in ExFHA women at rest or in response to an orthostatic challenge. Finally, it is acknowledged that regional MSNA in the lower limb may differ from SNA directed to other organs, such as the kidney and heart, and that cardiac vagal tone was not evaluated.

In conclusion, circulatory regulation of hypoestrogenic but otherwise healthy ExFHA women differs from eumenorheic women in several key respects. BP and HR are lower at rest and normal counter-regulatory responses to an orthostatic challenge are disrupted: plasma renin, angiotensin, and aldosterone fail to increase, and BP is defended by augmented reflex sympathetic vasoconstriction. A body of experimental and clinical work supports the concept that these alterations are a consequence of relative estrogen deficiency.

Perspectives
The present study adds to a body of literature describing altered cardiovascular function in exercise-trained women with FHA, including impaired conduit vessel function and increased regional vascular resistance. Similar hemodynamic changes have been documented in older sedentary hypoestrogenic postmenopausal women. We now report for the first time that compared with estrogen replete women, hypoestrogenic ExFHA women demonstrate (i) lower HR and BP during orthostatic stress as well as at rest; (ii) a shift toward sympathetic control of BP regulation during LBNP; and (iii) uncoupling of reflex sympathetic and RAAS responses to this hypotensive stimulus. These observations are strikingly similar to those identified previously in older postmenopausal women, suggesting estrogen deficiency may influence interactions between sympathetic activation and end-organ responsiveness. Studies reporting estrogen-mediated modulation of adrenergic receptor expression and sensitivity, norepinephrine synthesis and degradation, and postreceptor signaling cascades involved in signal transduction support this concept. The long-term consequences of neuro-humoral perturbations in youth are presently unknown, but it is now recognized that sympathetic activation contributes importantly to the development and progression of hypertension and cardiovascular disease, and it is of concern that studies in humans and nonhuman primates have identified estrogen deficiency as a result of FHA as a key contributing factor to early adulthood on cardiovascular risk, including hypertension.

Sources of Funding
P.J. Harvey was funded by a Pfizer Cardiovascular Independent Research Award (NRA 3840028). J.S. Floras holds the Canada Research Chair in Integrative Cardiovascular Biology. E. O’Donnell received doctoral funding from the Canadian Institute of Health Research. H. Murai was supported by the Bluma Appel International Fellowship of Mount Sinai Hospital. B.L. Morris was supported by an operating grant from the Heart and Stroke Foundation on Ontario.

Disclosures
None.

References
Novelty and Significance

What Is New?
- This is the first study to examine the effects of premenopausal estrogen deficiency on mechanisms of blood pressure regulation.
- We report for the first time that compared with their estrogen replete counterpart, estrogen-deficient physically active premenopausal women with functional hypothalamic amenorrhea (ExFHA) provide a unique model with which to examine the cardiovascular effects of estrogen deficiency. To date, the current study is the first to use this model of estrogen deficiency to examine the effects of hypoestrogenemia on blood pressure regulation.

What Is Relevant?
- The cardiovascular effects of estrogen deficiency per se in postmenopausal women are unclear, in part, because of the confounding effects of aging and oftentimes presence of comorbidities. Otherwise, healthy physically active premenopausal women with functional hypothalamic amenorrhea (ExFHA) provide a unique model with which to examine the cardiovascular effects of estrogen deficiency.
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Hypertension. 2015;65:1089-1095; originally published online March 16, 2015;
doi: 10.1161/HYPERTENSIONAHA.114.04976

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2015/03/16/HYPERTENSIONAHA.114.04976.DC1

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Discordant Orthostatic Reflex Renin-Angiotensin and Sympathoneural Responses in Premenopausal Exercising-Hypoestrogenic Women

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Short Title: Circulatory Regulation and Functional Amenorrhea
Online Data Supplement
Online Supplement

**Blood Sampling:** Blood samples were collected from a cannula placed in an antecubital vein at baseline and during the last minute of each stage of LBNP for determination of plasma renin and angiotensin II and serum aldosterone. Baseline blood samples for non-RAAS constituents were collected successfully in all subjects either from an indwelling forearm venous cannula or using standard venipuncture. Non-RAAS constituents included serum measures of 17 β-estradiol, progesterone, testosterone, follicle stimulating hormone, luteinizing hormone and sex hormone binding globulin (SHBG). On a separate day, 8-hour fasted serum free triiodothyronine (T3) was also assessed to provide an estimate of energy status, with low T3 levels indicating low energy status (i.e., energy deficiency) \(^1\). Free androgen index ([total testosterone/SHBG]*100) was calculated to provide an estimate of androgenicity \(^2\). All assays were run by the Toronto General Hospital Core Laboratory.

**Muscle Sympathetic Nerve Activity:** The nerve signals were amplified, filtered (bandwidth 700 to 2000 Hz), rectified, and integrated to obtain a mean voltage display of sympathetic nervous activity. A recording of MSNA was considered acceptable when the following criteria were met: i) spontaneous bursts of neural discharge synchronous with the heart rate; ii) no response to arousal stimuli or skin stroking; iii) an increase in nerve burst frequency with apnea; iv) a signal to noise ratio of 3:1 \(^3\). With the subject lying quietly, recordings were acquired at baseline and during each stage of LBNP. Signals were digitized (LabVIEW, National Instruments Corporation, Austin, TX, USA). Variables of interest included burst incidence (bursts/100 heart beats) and burst frequency (bursts/minute).
References