

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

Emma O'Donnell¹, Jack M Goodman^{1,2}, Susanna Mak², Hisa Murai³, Beverley L Morris³,
John S Floras^{2,3} and Paula J Harvey⁴.

¹Cardiovascular Research Laboratory, Department of Exercise Sciences, University of Toronto,
Toronto, Ontario, Canada, and Division of Cardiology at the ²Mount Sinai Hospital, ³Toronto
General Hospital, and ⁴Women's College Hospital, University of Toronto, Ontario, Canada.

Corresponding Author

Dr Paula J Harvey, BMBS, PhD, FRACP

Division of Cardiology, Women's College Hospital,

Department of Medicine, University of Toronto,

76 Grenville Street, Toronto, Ontario,

M5S 1B3, Canada

Tel: 416-323-7723

Fax: 416-323-6304

Email: Paula.harvey@wchospital.ca

Short Title: Circulatory Regulation and Functional Amenorrhea

Circulatory Regulation and Functional Amenorrhea

Total Word Count: 5528

Abstract Word Count: 267

Number of Figures: 1

ABSTRACT

Resting systolic blood pressure is lower in active hypoestrogenic women with functional hypothalamic amenorrhea. Our prior observations in normotensive postmenopausal women stimulated the hypotheses that compared to eumenorrheic women, their reflex renin-angiotensin-aldosterone system responses to an orthostatic challenge (graded lower body negative pressure) would be attenuated 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 \pm 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 burst incidence 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) women, 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 ExFHA, women ($P > 0.05$). Muscle sympathetic nerve activity burst incidence increased reflexively in both groups, but more so in ExFHA women ($P < 0.05$). 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.

INTRODUCTION

Functional hypothalamic amenorrhea (FHA) is a reversible cause of ovarian suppression in premenopausal women ¹. Its prevalence is markedly higher in recreationally and competitively exercising women and women with physically demanding jobs (~1-44%) than in sedentary women (~2-5%) ^{2,3}. Hypothalamic-pituitary-ovarian suppression in physically active women with FHA (ExFHA) has been related causally to energy deficiency (i.e., caloric deficit) due to high energy expenditure and insufficient energy intake (i.e., low caloric intake) ⁴.

Circulating estradiol concentrations in women with ExFHA are chronically low and resemble those observed in postmenopausal women and in men ^{5,6}. However in contrast to postmenopausal women, who are more likely than men to develop hypertension with advancing age ⁷, ExFHA women have *lower* resting systolic BP and heart rate (HR) than age-matched estrogen-replete physically active women ^{8,9}. 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 ¹⁰, whereas exogenous estrogen increases circulating levels of angiotensinogen, angiotensin I, renin, and angiotensin II ^{10,11} and decreases MSNA ¹².

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

Circulatory Regulation and Functional Amenorrhea

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^{8,9}, that women with ExFHA would have lower BP than eumenorrheic women BP both at rest and during LBNP. Based on our prior findings in postmenopausal women¹⁰, 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 behaviour, menstrual cycle, and medical health history. Eligibility criteria for the study included: 1) age 18 to 35 yrs; 2) good health determined by absence of chronic illnesses, including diabetes, hyperprolactinemia, poly-cystic ovarian syndrome, and thyroid disease; 3) stable menstrual status over the preceding 3 months defined as i) menstrual cycles between 25-35 days for menstruating women, or ii) complete absence of menses for at least 90 consecutive days in amenorrheic women; 4) taking no medications; 5) non-smoker; 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 or current clinical diagnosis of eating disorders; 9) participating in purposeful exercise for >2hrs per week; and 10) no other contraindications that would preclude participation in the study. The study was approved by the local University and Hospital Research Ethics Boards. All volunteers provided written informed consent.

Circulatory Regulation and Functional Amenorrhea

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 or more days ². Women reporting unpredictable menstrual cycles of variable length (i.e., 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 ⁸. In eumenorrheic women, ovulatory status was confirmed in the menstrual cycle prior to 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 0930-1030 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, prior to 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,

Circulatory Regulation and Functional Amenorrhea

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 (VO_2 peak) was measured using a metabolic cart during a progressive treadmill test to exhaustion (Moxus Modular VO_2 System, Applied Electrochemistry Inc., Pittsburgh, PA).

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 17β -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)¹³. Free androgen index ($[\text{total testosterone}/\text{SHBG}] \times 100$) was calculated to provide an estimate of androgenicity¹⁴. All assays were run by the Toronto General Hospital Core Laboratory.

Blood Pressure and Heart Rate: Systolic (SBP), diastolic (DBP), mean arterial BP (MAP) and HR were recorded from the left upper-arm using an automated device (Dinamap Pro 100, Critikon, USA). Brachial BP and HR were assessed at one minute intervals at baseline (three consecutive stable measures) and every minute throughout each stage of LBNP. The recordings

Circulatory Regulation and Functional Amenorrhea

acquired during the last four minutes of each LBNP stage were averaged to acquire a mean value for each LBNP stage. Continuous recordings of HR and BP were also acquired using lead II of an electrocardiogram and a photoplethysmographic device on the index finger (Portapres Model-2, Finapres Medical Systems BV, USA), respectively.

Muscle Sympathetic Nerve Activity: Multi-unit recordings of post-ganglionic MSNA were obtained with a unipolar tungsten electrode inserted selectively into a muscle-nerve fascicle of the right fibular (peroneal) nerve using previously described techniques¹⁵. 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 apnoea; iv) a signal to noise ratio of 3:1¹⁶. 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). 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 two groups known to differ with respect to cardiac frequency, the principal outcome variable was the heart rate-independent measure, MSNA burst incidence (bursts/100 cardiac cycles), which is derived from burst frequency (bursts/min) and heart rate.

Circulatory Regulation and Functional Amenorrhea

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 source¹⁰. 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 -10mmHg, -20mmHg and -40mmHg. Each LBNP stage was followed by 5-minutes of recovery (i.e., no LBNP). LBNP was terminated if SBP fell <80mmHg, pallor was observed, and/or subjective feelings of nausea, dizziness and/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 one-way analysis of variance (ANOVA). Within- and between-group analyses of responses to LBNP, including change (Δ) in measures compared to baseline, were determined using repeated measures ANOVA. 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 \pm SEM. A significance level of $P < 0.05$ was used to detect the differences for statistical procedures.

RESULTS

Circulatory Regulation and Functional Amenorrhea

Subject Characteristics: The two 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 two 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 1). 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 (burst/100 heart beats) did not differ ($P>0.05$) between groups (Table 2). Differences in baseline MSNA burst frequency (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, cannulization 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 1). 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. While between-group DBP values during LBNP did not differ ($P=0.45$), MAP trended ($P=0.06$)

Circulatory Regulation and Functional Amenorrhea

toward lower values in ExFHA. Δ HR, Δ SBP, Δ DBP and Δ MAP values during LBNP did not differ ($P>0.05$; all measures) between the groups. LBNP did not elicit pre-syncopal 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 1). 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 mmHg. Δ MSNA burst incidence, but not frequency, also demonstrated significantly greater ($P<0.05$) values in ExFHA versus ExOv women at -20 and -40 mmHg (Figure 1).

Within groups, LBNP elicited significant increases in renin ($P<0.01$), and Ang II ($P=0.01$), but not aldosterone ($P>0.05$; Figure 1). However, significant ($P<0.05$) between-group 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 1). 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 Δ 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

Circulatory Regulation and Functional Amenorrhea

This, to our knowledge, is the first study to report hemodynamics, efferent 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 with functional hypothalamic amenorrhea (ExFHA). This experiment yielded several novel findings. Compared with body mass index-, body composition-, age- and fitness-matched eumenorrheic women, hypoestrogenic women with functional hypothalamic amenorrhea demonstrated: i) similar tonic basal efferent sympathetic outflow to the lower limb and similar plasma renin, angiotensin II and aldosterone concentrations, yet lower resting heart rate and systolic blood pressure; ii) no reflex activation of renin, angiotensin II, or aldosterone in response to LBNP; and iii) consistently lower blood pressure and heart rate 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 upon augmented sympathoneural vasoconstrictor responsiveness to maintain blood pressure 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 less than 40 years old^{17, 18}. In both groups of women, graded LBNP elicited the anticipated reflex increase in MSNA burst incidence¹⁹. 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

Circulatory Regulation and Functional Amenorrhea

resting sympathetic discharge^{12, 20, 21}. 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 lower²² and similar²³ 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^{24, 25}. Combined oral hormonal contraceptives in premenopausal women²⁶ and transdermal estrogen therapy in postmenopausal women²⁷ 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 progesterone²¹ 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^{10, 28}, 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

Circulatory Regulation and Functional Amenorrhea

¹⁰. 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 ¹⁰. Estrogen has been shown to up-regulate angiotensinogen gene expression ²⁹, increase plasma angiotensin I and II concentrations, reduce angiotensin converting enzyme activity, alter plasma renin concentrations ^{10, 11}, 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 ³⁰. Estrogen stimulates endothelial nitric oxide and inhibits endothelin production ³¹. Thus, renal renin release may be diminished in estrogen deficient states.

RAAS activity has also been associated positively with circulating thyroid hormone levels ³². Thus, it is plausible that low triiodothyronine, as observed in ExFHA women in the current and in previous ^{8,9} studies, may also contribute to this low renin state. Considering the similarity of body mass index in our two 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 ²³ while sodium depletion and protein overfeeding can increase RAAS activity ³³. Mild negative energy balance may contribute to hyperadiponectinemia, hypoleptinemia, and low triiodothyronine ^{34, 35}. 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

Circulatory Regulation and Functional Amenorrhea

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 eumenorrheic 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 functional hypothalamic amenorrhea, including impaired conduit^{9,36} and resistance^{8,9} vessel function and increased regional vascular resistance^{8,9}. Similar hemodynamic changes have been documented in older sedentary hypoestrogenic postmenopausal women^{37,38}. 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 sympathoneural and RAAS responses to this hypotensive stimulus. These observations, likely a consequence of relative estrogen deficiency, are also strikingly similar to those identified previously in older postmenopausal women. The long-term consequences of these neuro-humoral perturbations in youth are presently unknown, but it is now recognized that sympathetic activation contributes importantly to cardiovascular disease

Circulatory Regulation and Functional Amenorrhea

development and progression¹⁷, and it is of concern that studies in humans and non-human primates have identified estrogen deficiency due to FHA as a key contributing factor to premenopausal coronary artery disease^{39,40}. Conversely, since elevated circulating renin and plasma angiotensin II levels in population⁴¹ and patient⁴² studies, respectively, are associated with increased cardiovascular risk, mortality and morbidity, absence of RAAS activation by simulated orthostasis may confer an element of cardiovascular protection. With growing numbers of young women participating in athletic activities⁴³, and a higher prevalence of hypothalamic estrogen deficiency in active than sedentary women², longitudinal studies following such women through later premenopausal years and into menopause should be undertaken to ascertain the cardiovascular risk associated with such neurohumoral perturbations during early adulthood.

Sources of Funding: PJH was funded by a Pfizer Cardiovascular Independent Research Award (NRA 3840028). JSF holds the Canada Research Chair in Integrative Cardiovascular Biology. EOD received doctoral funding from the Canadian Institute of Health Research. HM was supported by the Bluma Appel International Fellowship of Mount Sinai Hospital. BLM was supported by an operating grant from the Heart and Stroke Foundation on Ontario.

Conflicts of Interest: None

REFERENCES

1. Liu JH. Hypothalamic amenorrhea: Clinical perspectives, pathophysiology, and management. *Am J Obstet Gynecol.* 1990;163:1732-1736
2. Otis CL, Drinkwater B, Johnson M, Loucks A, Wilmore J. American college of sports medicine position stand. The female athlete triad. *Med Sci Sports Exerc.* 1997;29:i-ix
3. Friedl KE, Nuovo JA, Patience TH, Dettori JR. Factors associated with stress fracture in young army women: Indications for further research. *Mil Med.* 1992;157:334-338
4. Williams NI, Helmreich DL, Parfitt DB, Caston-Balderrama A, Cameron JL. Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. *J Clin Endocrinol Metab.* 2001;86:5184-5193
5. O'Donnell E, Goodman JM, Harvey PJ. Cardiovascular consequences of ovarian disruption: A focus on functional hypothalamic amenorrhea in physically active women. *J Clin Endocrinol Metab.* 2011;96:3638-3648
6. Carmina E, Stanczyk FK, Lobo RA. Laboratory assessment In: Strauss III JF, Barbieri RL, eds. *Yen and jaffe's reproductive endocrinology: Physiology, pathophysiology and clinical management* Philadelphia, PA: Saunders; 2014:822-858.
7. Reckelhoff JF. Sex steroids, cardiovascular disease, and hypertension: Unanswered questions and some speculations. *Hypertension.* 2005;45:170-174
8. O'Donnell E, Harvey PJ, Goodman JM, De Souza MJ. Long-term estrogen deficiency lowers regional blood flow, resting systolic blood pressure, and heart rate in exercising premenopausal women. *Am J Physiol Endocrinol Metab.* 2007;292:E1401-1409

Circulatory Regulation and Functional Amenorrhea

9. O'Donnell E, Goodman JM, Mak S, Harvey PJ. Impaired vascular function in physically active premenopausal women with functional hypothalamic amenorrhea is associated with low shear stress and increased vascular tone. *J Clin Endocrinol Metab.* 2014;99:1798-1806
10. Harvey PJ, Morris BL, Miller JA, Floras JS. Estradiol induces discordant angiotensin and blood pressure responses to orthostasis in healthy postmenopausal women. *Hypertension.* 2005;45:399-405
11. Ichikawa J, Sumino H, Ichikawa S, Ozaki M. Different effects of transdermal and oral hormone replacement therapy on the renin-angiotensin system, plasma bradykinin level, and blood pressure of normotensive postmenopausal women. *Am J Hypertens.* 2006;19:744-749
12. Vongpatanasin W, Tuncel M, Mansour Y, Arbique D, Victor RG. Transdermal estrogen replacement therapy decreases sympathetic activity in postmenopausal women. *Circulation.* 2001;103:2903-2908
13. Loucks AB, Heath EM. Induction of low-t3 syndrome in exercising women occurs at a threshold of energy availability. *Am J Physiol.* 1994;266:R817-823
14. Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, von Schoultz B, Hirschberg AL. Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. *Fertil Steril.* 2003;79:947-955
15. Wallin BG, Delius W, Hagbarth KE. Comparison of sympathetic nerve activity in normotensive and hypertensive subjects. *Circ Res.* 1973;33:9-21

Circulatory Regulation and Functional Amenorrhea

16. Notarius CF, Ando S, Rongen GA, Floras JS. Resting muscle sympathetic nerve activity and peak oxygen uptake in heart failure and normal subjects. *Eur Heart J*. 1999;20:880-887
17. Narkiewicz K, Phillips BG, Kato M, Hering D, Bieniaszewski L, Somers VK. Gender-selective interaction between aging, blood pressure, and sympathetic nerve activity. *Hypertension*. 2005;45:522-525
18. Ng AV, Callister R, Johnson DG, Seals DR. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension*. 1993;21:498-503
19. Sundlof G, Wallin BG. Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol*. 1978;274:621-637
20. Saleh MC, Connell BJ, Saleh TM. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res*. 2000;879:105-114
21. Minson CT, Halliwill JR, Young TM, Joyner MJ. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation*. 2000;101:862-868
22. Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS, Sinoway LI. Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol*. 2001;281:H2028-2035
23. Fu Q, Witkowski S, Okazaki K, Levine BD. Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. *Am J Physiol Regul Integr Comp Physiol*. 2005;289:R109-116

Circulatory Regulation and Functional Amenorrhea

24. Carter JR, Lawrence JE, Klein JC. Menstrual cycle alters sympathetic neural responses to orthostatic stress in young, eumenorrheic women. *Am J Physiol Endocrinol Metab*. 2009;297:E85-91
25. Fu Q, Okazaki K, Shibata S, Shook RP, VanGunday TB, Galbreath MM, Reelick MF, Levine BD. Menstrual cycle effects on sympathetic neural responses to upright tilt. *J Physiol*. 2009;587:2019-2031
26. Carter JR, Klein JC, Schwartz CE. Effects of oral contraceptives on sympathetic nerve activity during orthostatic stress in young, healthy women. *Am J Physiol Regul Integr Comp Physiol*. 2010;298:R9-R14
27. Fadel PJ, Wang Z, Watanabe H, Arbique D, Vongpatanasin W, Thomas GD. Augmented sympathetic vasoconstriction in exercising forearms of postmenopausal women is reversed by oestrogen therapy. *J Physiol*. 2004;561:893-901
28. Hinghofer-Szalkay HG, Vigas M, Sauseng-Fellegger G, Konig EM, Lichardus B, Jezova D. Head-up tilt and lower body suction: Comparison of hormone responses in healthy men. *Physiol Res*. 1996;45:369-378
29. Hilliard LM, Sampson AK, Brown RD, Denton KM. The "his and hers" of the renin-angiotensin system. *Current hypertension reports*. 2013;15:71-79
30. Wagner C, Jensen BL, Kramer BK, Kurtz A. Control of the renal renin system by local factors. *Kidney Int Suppl*. 1998;67:S78-83
31. Best PJ, Berger PB, Miller VM, Lerman A. The effect of estrogen replacement therapy on plasma nitric oxide and endothelin-1 levels in postmenopausal women. *Annals of internal medicine*. 1998;128:285-288

Circulatory Regulation and Functional Amenorrhea

32. Vargas F, Rodriguez-Gomez I, Vargas-Tendero P, Jimenez E, Montiel M. The renin-angiotensin system in thyroid disorders and its role in cardiovascular and renal manifestations. *J Endocrinol*. 2012;213:25-36
33. Swainson CP, Walker RJ. Renal haemodynamic and hormonal responses to a mixed high-protein meal in normal men. *Nephrol Dial Transplant*. 1989;4:683-690
34. O'Donnell E, De Souza MJ. Increased serum adiponectin concentrations in amenorrheic physically active women are associated with impaired bone health but not with estrogen exposure. *Bone*. 2010;48:760-767
35. O'Donnell E, Harvey PJ, De Souza MJ. Relationships between vascular resistance and energy deficiency, nutritional status and oxidative stress in oestrogen deficient physically active women. *Clin Endocrinol (Oxf)*. 2009;70:294-302
36. Zeni Hoch A, Dempsey RL, Carrera GF, Wilson CR, Chen EH, Barnabei VM, Sandford PR, Ryan TA, Gutterman DD. Is there an association between athletic amenorrhea and endothelial cell dysfunction? *Med Sci Sports Exerc*. 2003;35:377-383
37. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, Yeung AC, Creager MA. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. *Ann Intern Med*. 1994;121:936-941
38. Moreau KL, Donato AJ, Tanaka H, Jones PP, Gates PE, Seals DR. Basal leg blood flow in healthy women is related to age and hormone replacement therapy status. *J Physiol*. 2003;547:309-316
39. Bairey Merz CN, Johnson BD, Sharaf BL, Bittner V, Berga SL, Braunstein GD, Hodgson TK, Matthews KA, Pepine CJ, Reis SE, Reichel N, Rogers WJ, Pohost GM, Kelsey SF, Sopko G. Hypoestrogenemia of hypothalamic origin and coronary artery disease in

Circulatory Regulation and Functional Amenorrhea

- premenopausal women: A report from the nhlbi-sponsored wise study. *J Am Coll Cardiol*. 2003;41:413-419
40. Kaplan JR, Manuck SB. Ovarian dysfunction, stress, and disease: A primate continuum. *ILAR J*. 2004;45:89-115
41. de Boer RA, Schrotten NF, Bakker SJ, Mahmud H, Szymanski MK, van der Harst P, Gansevoort RT, van Veldhuisen DJ, van Gilst WH, Hillege HL. Plasma renin and outcome in the community: Data from prevend. *Eur Heart J*. 2012;33:2351-2359
42. Roig E, Perez-Villa F, Morales M, Jimenez W, Orus J, Heras M, Sanz G. Clinical implications of increased plasma angiotensin ii despite ace inhibitor therapy in patients with congestive heart failure. *Eur Heart J*. 2000;21:53-57
43. Javed A, Tebben PJ, Fischer PR, Lteif AN. Female athlete triad and its components: Toward improved screening and management. *Mayo Clin Proc*. 2013;88:996-1009

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 (FHA) demonstrate augmented muscle sympathetic nerve activity (MSNA) yet absence of renin-angiotensin-aldosterone system (RAAS) activation during orthostatic stress.

What is relevant?

The cardiovascular effects of estrogen deficiency *per se* in postmenopausal women are unclear, in part due to the confounding effects of aging and oftentimes presence of co-morbidities.

Otherwise healthy physically active premenopausal women with FHA (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.

Summary

The present study demonstrates a shift toward greater sympathetic and a withdrawal of RAAS support of arterial BP in ExFHA women during hypotensive stimuli. These results indicate altered neurohumoral control of blood pressure in ExFHA women, suggesting an important role for endogenous estrogen in blood pressure regulation in premenopausal women.

Circulatory Regulation and Functional Amenorrhea

Key words: blood pressure, estrogens, exercise, renin-angiotensin system, sympathetic nervous system

Circulatory Regulation and Functional Amenorrhea

Figure 1: 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 (A), mean arterial blood pressure (B), heart rate (C); serum measures of renin (D), angiotensin II (E), and aldosterone (F); and muscle sympathetic nerve activity, 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 LBNP, GROUP, and interactions between these factors (LBNP x GROUP) are reported separately on each graph. Values are mean \pm SEM.

Table 1. Baseline subject characteristics.

	ExOv	ExFHA	<i>P</i>
	(n=17)	(n=12)	
Age (yrs)	24±1	25±1	0.308
Height (cm)	166±2	168±1	0.316
Weight (kg)	57.5±1.9	58.4±2.1	0.761
BMI (kg/m²)	20.9±0.5	20.7±0.7	0.828
Body fat (%)	23.5±1.8	21.8±2.1	0.592
Lean mass (kg)	41.9±1.6	44.2±1.7	0.377
VO₂ peak (ml/kg/min)	47.1±1.3	46.3±1.9	0.666
Estradiol (pmol/L)	154±24	87±13	0.045
Progesterone (nmol/L)	1.6±0.2	1.4±0.2	0.634
Free T3 (pmol/L)	4.2±0.2	3.6±0.1	0.017
Testosterone (nmol/L)	2.1±0.1	2.4±0.3	0.246
SHBG (nmol/L)	51±3	51±4	0.982
FAI (au)	4.6±0.3	4.8±0.7	0.762
LH (IU/L)	4.1±0.9	1.6±0.4	0.013
FSH (IU/L)	7.6±2.4	5.4±0.8	0.318
Amenorrhea (days)[†]	-	1061±319	-

Values mean±SEM.

BMI, body mass index; FAI, free androgen index; FSH, follicle stimulating hormone; LH, leutinizing hormone; SHBG, sex hormone binding globulin; T3, triiodothyronine; VO₂ peak, peak oxygen uptake.

[†] Range 150-3132 days.

Table 2. Hemodynamic responses of the groups at baseline and during graded LBNP (mmHg).

	Ba	-10	-20	-40
SBP (mmHg)				
<i>ExOv</i>	105±2	101±2 [*]	100±2 [*]	98±1 [*]
<i>ExFHA</i>	94±2 [†]	93±2 [†]	92±2 [†]	90±3 ^{†*}
DBP (mmHg)				
<i>ExOv</i>	60±1	56±2 [*]	54±2 [*]	53±2 [*]
<i>ExFHA</i>	58±6	56±2	53±2 [*]	51±2 [*]
MAP (mmHg)				
<i>ExOv</i>	75±1	71±2 [*]	69±2 [*]	68±2 [*]
<i>ExFHA</i>	70±2 [†]	68±2	66±2 [*]	64±2 [*]
PP (mmHg)				
<i>ExOv</i>	45±2	45±2	45±2	45±2
<i>ExFHA</i>	36±2 [†]	36±2 [†]	39±2 [†]	39±2 [†]
HR (b/min)				
<i>ExOv</i>	56±2	58±2	62±3 [*]	72±4 [*]
<i>ExFHA</i>	47±2 [†]	48±2 [†]	49±2 [†]	60±2 ^{†*}

Values are mean±SEM.

Ba, baseline; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

* Significantly different from baseline within-groups, $p < 0.05$.

† Significantly different between-groups for given LBNP, $p < 0.05$.

Table 3. Neurohumoral measures at baseline and during graded LBNP (mmHg).

	Ba	-10	-20	-40
Renin (pmol/L) *				
<i>ExOv</i>	8.0±2.0	7.4±1.8	8.7±2.0	12.5±2.5 [†]
<i>ExFHA</i>	4.0±0.8	4.2±0.8	4.3±0.7 [‡]	4.8±0.9 [‡]
Ang II (pmol/L) *				
<i>ExOv</i>	3.9±0.8	4.1±0.9	5.3±1.4 [†]	8.6±1.9 [†]
<i>ExFHA</i>	2.3±0.3	2.6±0.4	2.1±0.3 [‡]	2.0±0.4 [‡]
Aldo (pmol/L) *				
<i>ExOv</i>	88±16	74±8	77±9	134±30
<i>ExFHA</i>	132±32	117±32	104±25	96±27
MSNA (frequency) §				
<i>ExOv</i>	8±2	10±2	13±2 [†]	22±3 [†]
<i>ExFHA</i>	10±2	13±3	18±2 [†]	27±2 [†]
MSNA (incidence) §				
<i>ExOv</i>	15±3	18±4	22±4 [†]	33±4 [†]
<i>ExFHA</i>	22±5	28±6	37±4 ^{‡,†}	45±4 ^{‡,†}

Values are mean±SEM.

Ba, baseline; aldo, aldosterone; ang II, angiotensin II; frequency, burst frequency measuring number of bursts per minute; incidence, burst incidence measuring number of bursts per 100 heart beats; 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

Figure 1.

