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Indexes of Aortic Wave Reflection are not Augmented in Estrogen Deficient Physically Active Premenopausal Women

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Running Title: Arterial Compliance in Amenorrheic Women

Key words: amenorrhea; arterial compliance; augmentation index; estrogen deficiency; exercise.

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ABSTRACT

Background: Hypoestrogenemia due to menopause is associated with increased cardiovascular disease risk, in part due to elevated indexes of aortic wave reflection (AWRI) and central (aortic) blood pressure. We sought to investigate whether AWRI and central blood pressure are also augmented in hypoestrogenic exercise-trained premenopausal women with functional hypothalamic amenorrhea (ExFHA). **Methods:** In age- (pooled mean $\pm$ SEM, 24 $\pm$ 1 years), BMI- (21 $\pm$ 1 kg/m²), and cardiorespiratory fitness-matched (45 $\pm$ 2 ml/kg/min) eumenorrheic ovulatory (ExOv; n=11) and ExFHA women (n=10), we assessed aortic blood pressure and wave form characteristics (augmentation index and wave reflection amplitude) obtained from radial pressure waves (applanation tonometry). Doppler ultrasound determined cardiac output (CO) and total peripheral resistance (TPR). Measures were recorded before and one-hour after 45 minutes of moderate intensity exercise to determine the influence of exercise-induced increases in nitric oxide. **Results:** Pre-exercise, Alx75, central systolic BP (SBPc), and CO were lower ($p<0.05$) and TPR higher ($p<0.05$) in ExFHA. Post-exercise, Alx75 was unchanged ($p>0.05$) in ExFHA but was lowered ($p<0.05$) in ExOv. Both groups demonstrated increased CO, and lowered SBPc and TPR, yet TPR remained higher ($p<0.05$), and CO and SBPc lower ($p<0.05$) in ExFHA. **Conclusions:** Despite hypoestrogenemia, functional compliance of the central arteries and central BP are not augmented, yet TPR is higher, in ExFHA versus ExOv. An acute bout of dynamic exercise did not alter Alx75 in ExFHA, suggesting blunted vascular responsiveness to exercise-induced increases in nitric oxide, possibly due to augmented vascular tone. These findings have relevance in understanding the vascular consequences of hypoestrogenemia during the premenopausal years.

INTRODUCTION

Functional hypothalamic amenorrhea (FHA), a reversible and common cause of estrogen deficiency in premenopausal women ¹, is characterized by altered hypothalamic–pituitary–ovarian axis function causing cessation of menses and marked hypoestrogenemia ².

Estrogen deficiency due to menopause is associated with increased cardiovascular disease risk ^{3,4} in part due to the loss of estrogen-mediated increases in endothelium-derived nitric oxide (NO), a potent vasodilator and cardioprotective molecule ⁵. Accordingly, estrogen deficiency associated with FHA is independently associated with the premature development of coronary artery disease ^{3,4}.

Prevalence of FHA is significantly higher among physically active women, including recreational and competitive athletes, and those with physically demanding jobs (~1-44%), compared with sedentary women (~2-5%) ^{6,7}. FHA in physically active women (ExFHA) has been causally linked to energy deficiency (i.e., negative caloric balance) that is associated with high energy expenditure combined with inadequate caloric intake ⁸. Estrogen deficiency-associated cardiovascular perturbations in ExFHA include impaired brachial artery endothelium-dependent and -independent function, low regional blood flow and elevated vascular resistance, discordant neurohumoral blood pressure responses to simulated orthostatic stress, and altered autonomic modulation of heart rate ⁹⁻¹⁵. The long-term clinical consequences of these perturbations in ExFHA are unknown.

Similar to estrogen, exercise also exerts beneficial effects on the vasculature. Exercise-induced increases in blood flow elicits increases in shear stress resulting in increased production of endothelium-derived NO ¹⁶. In keeping with this, acute dynamic exercise is associated with improved endothelium-dependent function and decreased regional vascular resistance in ExFHA ¹⁰. However, vascular resistance remains higher and vascular function, while improved, is not 'restored' to a level observed in their eumenorrheic counterpart, suggesting augmented vascular tone and/or altered structure in ExFHA.

Changes in vascular structure are paralleled by decreases in arterial compliance ¹⁷. Decreased arterial compliance causes reflected pressure waves from the periphery to travel toward the heart at high velocity, arriving early at the aorta during systole instead of diastole.

The early arrival of the pressure wave increases central (aortic) pressures and afterload ¹⁸. Thus, augmented indexes of aortic wave reflection (AWRI) and elevated central blood pressure are associated with increased risk of developing heart disease and experiencing adverse cardiovascular events ¹⁸. In postmenopausal women, AWRI and central blood pressure are elevated compared with age-matched men and younger premenopausal women ¹⁹. Whether estrogen deficiency in young exercise-trained women with FHA is associated with increased AWRI or central blood pressure is unknown. Accordingly, we sought to examine indexes of AWRI, namely augmentation index and augmentation pressure, and central blood pressure in ExFHA and compare with values observed in eumenorrheic physically active women (ExOv), both before and one-hour post-exercise. We hypothesized that compared with ExOv, ExFHA would demonstrate higher baseline AWRI and central blood pressure. It was also postulated that after an acute bout of dynamic exercise, a known nitric oxide stimulus ¹⁶, AWRI and central blood pressure would be lowered in ExFHA, similar to values observed in ExOv.

METHODS

Subjects: Participants (n=21) were recruited by posters targeting physically active women for a study on women's health. Screening procedures included general questionnaires on exercise, eating behaviour, menstrual cycle, and medical health. Eligibility criteria for the study included, 1) age 18 to 35 yrs; 2) good health determined by a medical examination; 3) no chronic illness, including diabetes, hyperprolactinemia, poly-cystic ovarian syndrome, and thyroid disease; 4) stable menstrual status over the preceding 3 months (i.e., absence of menses for at least 90 or more consecutive days or menstrual cycles between 25-35 days); 5) non-smoker; 6) not currently dieting and weight stable for the preceding 3 months, as determined by self-report; 7) no history or current clinical diagnosis of eating disorders; 8) absence of hormonal contraceptive use for at least 6 months; 9) no medications; 10) not pregnant or breastfeeding; and 11) no other contraindications that would preclude participation in the study. The study protocol conforms to the ethical guidelines of the 1975

Declaration of Helsinki and was approved by the institutional committee on human research by the Ethics Review Board at the University of Toronto and the University Health Network. All participants signed an approved informed consent document.

Experimental Design: Participants were recruited consecutively over three years to participate in a larger cross-sectional study examining the cardiovascular consequences of estrogen deficiency in premenopausal women. Fifty-seven women participated in the larger study, which was comprised of three smaller studies. Participants enrolled in 1-3 of these smaller studies. We have previously published data on subject characteristics, and heart rate and blood pressure for many of the 57 women ^{10,11}. However, for the first time we report AWRI, central blood pressure, cardiac output and total peripheral resistance, both before and one hour after exercise, in 21 of the 57 women.

Exercise and Ovulatory Status: “Exercising” was defined as self-reported purposeful exercise of more than 2 hours per week ^{10,13}. Duration (minutes) of exercise training across a typical training week was used to provide an estimate of weekly training volume. An objective exercise-status criterion measure of peak aerobic capacity of ≥ 40 ml/kg/min was also used to reflect exercising status ^{10,13}.

In eumenorrheic women, ovulatory status was confirmed during the menstrual cycle prior to testing. Ovulation was detected using an industry recognized ovulation hormone kit (Clearblue Easy ovulation test, Unipath Diagnostics, Waltham, MA).

Study Groups: Two groups of age-, fitness-, and body composition-matched physically active women were studied: women with eumenorrheic ovulatory menstrual cycles (ExOv; n=11), and FHA women (ExFHA; n=10). FHA was defined as cessation of menses for at least 90 consecutive days ⁶. Women reporting unpredictable menstrual cycles of variable length (i.e., oligomenorrhea) were excluded.

Anthropometric Measures: Total body mass (recorded to the nearest 0.5 kg) and height (recorded to nearest 1.0 cm) were determined using a physician's balance scale (Detecto, Webb City, MO). Body mass index (BMI, kg/m²) and body surface area (BSA; $[W^{0.425} \times H^{0.725}] \times 0.007184$) were calculated, with W being weight in kg, and H height in centimeters.

Peak Aerobic Capacity: Peak aerobic capacity (VO₂ peak) was measured once by a progressive treadmill test to volitional exhaustion on a separate day prior to the study day. Breath-by-breath expired gas samples were collected continuously to determine ventilation and oxygen consumption using a calibrated automated metabolic cart (Moxus Modular VO₂ System, Applied Electrochemistry Inc., Pittsburgh, PA).

Body Composition: Dual-energy x-ray absorptiometry (DXA; Prodigy, General Electric Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069) was utilized to determine body composition by a qualified operator. Percent body fat and lean mass were determined.

Protocol: All testing took place in the morning, starting between 08:30-09:30 hrs in a quiet ambient room (22-24 °C). Volunteers were 8 hours fasted and had abstained from exercise and caffeine for 24 hours and alcohol 12 hours prior to testing. All measures were obtained during the early follicular phase (days 2-6) in menstruating women, and on a random day for amenorrheic women. Standard venipuncture techniques were used to collect blood samples. After 20 minutes of quiet supine rest, baseline augmentation index (AIx) and augmentation pressure (AP) were recorded in duplicate, and heart rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) in triplicate. Once completed, participants exercised (brisk treadmill walking) for 45 minutes at 60% of their peak aerobic capacity (determined on a separate day). HR corresponding to 60% of maximal oxygen consumption was used to monitor exercise intensity. To avoid sensations of hunger or hypoglycemia post-exercise, subjects consumed a standard light low-fat lunch

immediately after exercise. All measures were repeated in the supine position 60 minutes post-exercise.

Pulse Wave Analysis: Indexes of aortic wave reflection (AWRI) were assessed in duplicate using applanation tonometry methods (SphygmoCor®, AtCor Medical Pty Ltd, West Ryde, NSW, Australia) to assess the pressure wave measured non-invasively on the right radial artery. Continuous recordings of blood pressure and heart rate were ceased 15 minutes prior to, and throughout assessment of, AWRI. Measures were recorded by an experienced single operator (EOD). Only recordings with an operator index of 80% or above were accepted for analyses. Radial artery pressure waveforms were calibrated from brachial artery measurements of BP. Using a validated generalized transfer function, a corresponding central (aortic) pressure waveform was generated¹⁸. Indexes of interest included: aortic blood pressure (central systolic, diastolic, mean arterial and pulse pressure; SBPc, DBPc, MAPc, and PPc, respectively), aortic augmentation pressure (AP; mmHg), aortic augmentation index (Alx; %), and due to Alx being inversely correlated with heart rate (HR)²⁰, Alx was also adjusted for a HR of 75 beats/min (Alx75; %). Alx assesses the timing of the reflected wave at the aorta as it relates to the onset of systole, with arrival during diastole resulting in low or negative Alx values, and arrival during systole resulting in elevated positive Alx values. This timing is linked to arterial compliance, such that higher Alx values indicate lower arterial compliance, or higher 'stiffness', and *vice versa*. Alx is calculated as $AP/PP \times 100$ to give a percentage¹⁸, where PP (pulse pressure; mmHg) is calculated as SBP minus DBP. AP is calculated as the difference between the second and the first systolic shoulder of the pressure wave form, representing peak systolic pressure and the inflection point which is indicative of SBP at the moment of arrival of the peripherally generated reflected wave (wave travelling toward the heart) at the aorta, respectively¹⁸. PP amplification was calculated as the peripheral-to-central PP ratio.

Hemodynamic Measures: Average (mean of three measures) brachial SBP, DBP, and MAP ($1/3$ pulse pressure + DBP) and HR were recorded using an automated device

(Dinamap Pro 100, Critikon, USA) on the left upper-arm. Continuous BP and HR were also recorded on a beat-to-beat basis using a photo-plethysmographic method on the middle digit of the right hand (Finapres, Ohmeda, Madison, WI).

The maximum instantaneous aortic flow velocity was measured in the ascending aorta during held expiration using continuous wave Doppler (1.5-4.0 MHz) directed through the suprasternal window ²¹. SV was calculated from the product of the mean time velocity integral and the cross-sectional area of the aortic annulus ²¹. CO was calculated from the product of SV and HR. TPR and total peripheral conductance (TPC) were derived from MAP and CO.

SV and CO were indexed to adjust for body surface area (BSA, m²; calculation shown in Anthropometric Measures). The indexed data were calculated as follows: SV index, SVI (ml/min/m²) = SV/BSA; and cardiac index, CI (L/min/m²) = CO/BSA.

Blood Sampling: Blood samples were collected from the antecubital vein using standard venipuncture techniques for determination of serum measures of 17 β -estradiol, progesterone, total testosterone, sex hormone binding globulin, and free triiodothyronine (T3). Free androgen index ([total testosterone/SHBG]*100) was calculated to assess androgenicity. All assays were run by the Toronto General Hospital Core Laboratory.

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 exercise compared to baseline were determined using repeated measures ANOVA. When assumptions of sphericity were violated, the Greenhouse-Geisser correction was used. Using pooled data, and using independently grouped data, Pearson's correlational analyses were used to determine significant linear independent associations between AWRI and hormonal and hemodynamic variables of interest. Data were analyzed using packaged software (SPSS version 22;

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

Subject Characteristics: The study groups did not differ ($p > 0.05$) in age, height, weight, body mass, body composition, cardiorespiratory fitness and serum progesterone levels (Table 1). Volume of weekly exercise training was also similar ($p > 0.05$) between groups (5.2 ± 0.6 vs. 4.8 ± 0.5 hours, ExOv vs. ExFHA, respectively). Consistent with energy deficiency as the primary cause of amenorrhea in exercising premenopausal women⁸ circulating levels of triiodothyronine were lower ($p < 0.05$) in ExFHA. Estradiol was also lower ($p < 0.05$) in ExFHA.

Baseline Pulse Wave Analysis and Hemodynamics: Between groups, resting AP and Alx did not differ ($p > 0.05$). In contrast, Alx75 was lower ($p < 0.05$) in ExFHA (Table 3). Resting brachial and central BP are shown in Table 2 and 3, respectively. Brachial and central SBP and PP were lower ($p < 0.05$) in ExFHA. Baseline brachial, but not central, MAP was lower ($p < 0.05$) in ExFHA. Brachial and central DBP, and PP amplification did not differ between the groups ($p > 0.05$).

Within groups, central SBP and PP were lower ($p < 0.05$) than that measured in the brachial artery. In contrast, brachial versus central DBP and MAP did not differ ($p > 0.05$).

Resting CO, CI, TPC and HR were lower ($p < 0.05$) and TPR higher ($p < 0.05$) in ExFHA (Table 2). Using central measures of MAP to calculate TPR did not alter ($p > 0.05$) the finding of higher resting TPR in ExFHA. SV and SVI did not differ ($p > 0.05$) between the groups.

Post-Exercise Pulse Wave Analysis and Hemodynamics: One-hour post exercise Alx, Alx75 and AP were decreased in ExOv, but were unaltered in ExFHA, such that none of these parameters differed between groups ($p > 0.05$). Exercise lowered brachial DBP and MAP, and increased brachial PP, in both groups ($p < 0.05$; Table 2). In contrast, brachial SBP was not altered by exercise in either group ($p > 0.05$). Conversely, exercise lowered central

SBP and MAP, yet did not alter PP in either group ($p < 0.05$; Table 3). Central DBP was unaltered by exercise ($p > 0.05$) in ExOv yet was decreased ($p < 0.05$) in ExFHA. PP amplification was higher ($p < 0.05$) in ExOv (1.70) than in EFHA (1.56), with PP increasing from baseline in ExOv ($p < 0.05$) but remaining unaltered ($p > 0.05$) in ExFHA.

Both groups demonstrated an increase in CO and CI ($p < 0.05$) and a decrease in TPR ($p < 0.05$) compared with pre-exercise values. However, CO and CI remained lower ($p < 0.05$) and TPR higher ($p < 0.05$) in ExFHA. SV and SVI were unaltered one-hour post-exercise ($p > 0.05$) and did not differ ($p > 0.05$) between the groups.

Using delta (post-exercise minus pre-exercise) values, PP (-1.8 vs 2.2 mmHg), AP (-3.6 vs -1.1 mmHg) and Alx (-12.0 vs 4.4%) significantly differed ($p < 0.05$) between ExOv vs ExFHA, respectively. In contrast, Alx75 (-6.5 vs 0.4%; $p = 0.082$), CO (0.84 vs 0.45 L/min; $p = 0.05$) and TPR (-291 vs 223 dynes-sec/cm⁵; $p = 0.240$) did not statistically differ between ExOv vs ExFHA, respectively.

Correlates of Pulse Wave Analyses: At rest, in ExFHA women only, Alx, Alx75 and AP were positively associated ($p < 0.05$) with TPR ($r = 0.906$, $r = 0.701$, and $r = 0.738$, respectively; Figure 1). Alx and AP ($p < 0.05$), but not Alx75 ($p = 0.064$), were inversely associated with CO ($r = -0.866$ and $r = -0.738$) in ExFHA women. Alx was inversely correlated with HR in both ExFHA ($r = -0.723$, $p < 0.05$) and ExOv women ($r = -0.790$, $p < 0.05$). Central PP was positively correlated with both estradiol ($r = 0.759$, $p < 0.05$) and triiodothyronine ($r = 0.693$, $p < 0.05$) in ExFHA only.

Post-exercise, Alx, Alx75 and AP remained positively associated ($p < 0.05$) with TPR ($r = 0.705$, $r = 0.679$, and $r = 0.710$, respectively) in ExFHA. Alx and AP also remained inversely associated ($p < 0.05$) with CO ($r = -0.816$ and $r = -0.814$) in ExFHA. Alx was not correlated ($p > 0.05$) with HR in either group.

Using delta (post-exercise minus pre-exercise) values for AWRI and hemodynamic measures, no correlations ($p > 0.05$) were observed for pooled or group data.

DISCUSSION

Our novel data suggest that despite estrogen deficiency, young physically active women with FHA do not demonstrate augmented indexes of aortic wave reflection, namely $Alx75$ and central BP, compared with their eumenorrheic counterpart. Additionally, CO is also lower and TPR higher, in these women. One-hour after an acute bout of dynamic exercise, a known NO stimulus, $Alx75$ is unaltered yet central blood pressures and CO remained lower, and TPR higher, in physically active women with FHA. Collectively, these findings suggest that aortic stiffness is not increased in estrogen deficient ExFHA women, rather their functional arterial compliance appears to be well preserved. However, unaltered post-exercise AWRI in ExFHA suggests their vascular responsiveness to exercise-mediated increases in NO may be impaired, or possibly mitigated, by vasoconstricting factors such as augmented vascular tone.

Pre-Exercise Aortic Wave Reflection

For the first time we report that in comparison to eumenorrheic ovulatory physically active women, estrogen deficient ExFHA women demonstrate lower $Alx75$, suggesting augmented functional arterial compliance of the central arteries. However, our finding of low AWRI contrast that of a recent study reporting similar resting central (aortic) and peripheral pulse wave velocity, a direct marker of arterial stiffness (and its inverse, arterial compliance), in amenorrheic and eumenorrheic athletes ²². Causes of disparity are unclear, but may be related in part to differences in arterial assessment. Pulse wave velocity assesses the speed of the propagated pulse wave through the arterial tree. Aortic wave reflection assesses the amplitude and the timing of the reflected wave arriving at the aorta from the periphery. While both measures provide an estimate of the intrinsic elastic properties of the central arteries ¹⁸, it is unknown if AWRI are correlated with pulse wave velocity measures in ExFHA.

Low AWRI in estrogen deficient ExFHA women seems paradoxical given the previous findings of impaired brachial artery flow-mediated dilation ^{10,14,15} and blunted endothelium-independent vasodilation in response to exogenous NO ^{10,15}. Impaired flow-mediated dilation in ExFHA has been postulated to be due in part to diminished estrogen-mediated

production of endothelium-derived NO^{10,14,15}. Estrogen plays a key role in vascular regulation through the rapid, non-genomic increase in production and bioavailability of NO via activation of the PI3K/Akt pathway¹⁰. As a potent vasodilator, NO modulates both endothelial function and AWRI²³. Accordingly, studies report that serum levels of NO closely parallel those of estrogen across the menstrual cycle²⁴. During the high estrogen phase of the menstrual cycle, elevated levels of NO are associated with augmented brachial artery endothelium-dependent flow-mediated dilation, and lowered Alx75 and forearm vascular resistance, compared with the low-estrogen phase of the menstrual cycle²⁴. Estrogen deficiency in postmenopausal women is also associated with low circulating NO levels²⁵, impaired endothelial function, and increased vascular resistance and arterial stiffness²⁶⁻²⁸, with estrogen therapy improving each of these parameters, including NO²⁵⁻²⁸. Thus, low rather than elevated AWRI in estrogen deficient ExFHA suggests factors other than hypoestrogenemia are at play.

Interplay between blood pressure and arterial compliance may contribute to the finding of low AWRI in ExFHA. Pulse pressure is a recognized surrogate of arterial compliance, with higher pulse pressure being associated with lower arterial compliance²⁹. Lower distending pressures in the vasculature due to low arterial BP predominantly activates elastin fibers compared with greater collagen fiber recruitment at higher distending pressures³⁰. As such, with increasing distending pressures the vessels become 'stiffer', or less compliant. It is possible, therefore, that low central pressures in ExFHA may result in low arterial distension, which could in turn augment elastin activation resulting in increased arterial compliance, as suggested by their low Alx75. This postulate awaits investigation.

Pre-Exercise Hemodynamics

Compared with ExOv, ExFHA demonstrate lower resting CO in association with lower resting HR. Even when indexed to body size, CO remained lower in ExFHA. CO is known to be a tightly regulated process that meets the balanced oxygen and substrate demands of the tissues³¹. Thus, low CO in ExFHA may reflect diminished substrate demands of the tissues. This notion is consistent with studies reporting lower whole-body³² and lower limb¹²

metabolic demand, and lower regional blood flow ¹³ in amenorrheic athletes compared with their eumenorrheic counterpart. These allostatic adaptations are postulated to conserve energy in the face of energy deficiency ^{12,32}.

Elevated TPR in ExFHA is consistent with previous studies reporting increased vascular resistance in the lower limb in physically active women with FHA ^{10,12,13}. While the role of estrogen deficiency *per se* is unclear, elevated TPR in ExFHA is consistent with findings reported in postmenopausal women ²⁷. Increased vascular tone in postmenopausal women is directly associated with increased efferent muscle sympathetic nerve outflow in the lower limb skeletal muscle vascular bed ³³. Loss of the sympatho-inhibitory effects of estrogen due to menopause are postulated to play a role ^{27,33}. In keeping with this, hypoestrogenic ExFHA women demonstrate augmented muscle sympathetic nerve activity during simulated orthostatic stress compared with their eumenorrheic counterpart ¹¹. However, the relationship between estrogen levels, TPR and muscle sympathetic nerve activity in ExFHA remain unknown.

In response to energy deficiency, ExFHA demonstrate numerous metabolic and hormonal perturbations, including low levels of triiodothyronine (T3) ^{9,10,12,13,34}. T3, the physiologically active form of thyroid hormone, is a classic indicator of nutritional status, but it is also known to exert cardiovascular effects. Studies report increases in HR and CO, and decreases in vascular resistance in response to T3 administration in patients with low T3 ^{35,36}. In keeping with this in part we report lower T3, lower resting HR and CO, and higher TPR in ExFHA versus ExOv. However, in the current study T3 was not correlated with CO or TPR, but, consistent with previous studies ^{9,12}, was positively associated with HR in ExFHA. Collectively, our findings suggest low T3 in ExFHA does not independently play a role in central hemodynamics, but may play a possible role in cardiac autonomic modulation of HR.

Post-exercise Aortic Reflection and Hemodynamics

Alx75 was unchanged in ExFHA women one-hour post-exercise, but was decreased in ExOv, such that it no longer differed from that seen in ExFHA. Consistent with this, we also

observed that post-exercise central PP no longer differed between the groups. These findings suggest functional arterial compliance was unaltered by exercise in ExFHA. This observation is inconsistent with the well established effects of exercise on vascular function and NO production ¹⁶. Through the same PI3K/Akt biochemical pathway as estrogen, but through different mechanisms of activation, exercise increases the production of endothelium-derived NO and other vasoactive substances due to increases in blood flow and consequent increases in shear stress ¹⁶. Given that NO has been demonstrated to modulate arterial compliance ³⁷, unaltered post-exercise Alx75 in ExFHA suggests impaired smooth muscle cell responsiveness to NO and/or arterial remodeling. However, in the absence of assessment of NO levels in the current study, we are unable to confirm this. Notwithstanding, this notion is supported in part by previous studies reporting improved, but not 'restored' post-exercise brachial artery flow-mediated dilation ¹⁰ and blunted endothelium-independent vasodilatory response to exogenous NO in ExFHA ^{10,15}. Mitigating factors that attenuate the vasodilatory response to NO may also be contributing to altered post-exercise vascular responsiveness in ExFHA women. Such factors might include augmented post-exercise muscle sympathetic nervous system activity and/or increased sympathetic vascular transduction. In postmenopausal women, estrogen deficiency-mediated loss of vascular beta-adrenergic receptor sensitivity is reported to be associated with augmented vascular transduction of sympathetic outflow into vascular resistance ³³. The influence of estrogen deficiency in ExFHA on vascular beta-adrenergic sensitivity and sympathetic vascular transduction remain to be determined.

Limitations

The current study assessed indexes of aortic wave reflection as a surrogate of arterial stiffness. While this is a validated method ¹⁸, use of other established techniques such as pulse wave velocity, beta-stiffness index, or systemic arterial compliance could have provided us with greater insight into the structural properties of the vasculature in ExFHA women. It is possible that low AWRI in ExFHA, both before and after exercise, may have been influenced by exercise training status. However, both ExOv and ExFHA presented with

similar aerobic capacity and reported similar volumes of endurance exercise training. Thus, exercise training likely does not play a role in our findings. The current study incorporates small-sample sizes and as such our findings should be interpreted with caution. Despite the known effects of dynamic exercise on the production of endothelium-derived NO¹⁶, we cannot confirm that NO was increased post-exercise in both study groups. We were also unable to definitively establish the independent effects of estrogen deficiency on AWRI in ExFHA, in part due to the confounding effects of energy deficiency and exercise training, both of which are known to independently influence vascular health^{38,39}. It is also possible a higher exercise intensity was required to elicit changes in AWRI in ExFHA.

Conclusion

Despite the known biology of estrogen, and previous studies reporting impaired endothelium-dependent and -independent function in ExFHA, we report lower indexes of aortic wave reflection in association with augmented TPR in ExFHA ExOv. We also report lower CO in association with lower heart rate, and lower central blood pressures in ExFHA. After an acute bout of dynamic exercise, aortic wave reflection, specifically Alx75, was unaltered, central blood pressure was lowered, and while TPR was also lowered, it remained elevated in ExFHA. Although the exact role of estrogen deficiency in these observations are unclear, these findings suggest low arterial pressures in ExFHA may contribute to vascular phenotypical changes that elicit augmented arterial compliance. Increased vascular tone in ExFHA may act as a compensatory mechanism to maintain perfusion pressure and/or blood flow. Impaired or mitigated vascular responsiveness to exercise-mediated increases in NO and/or altered arterial structure may also play a role. While the clinical relevance of our findings remain to be determined, hypoestrogenemia due to FHA in untrained women has previously been linked to accelerated atherosclerosis³. Whether favorably altered timing of aortic wave reflections mitigates this potential cardiovascular risk in ExFHA^{10,14,15}, remains to be determined. Given that young healthy women are not typically thought of as 'at risk' by cardiologists and other physicians, and as a result are more likely to be overlooked⁴⁰,

investigations into the long-term cardiovascular consequences of hypoestrogenemia in physically active premenopausal women are of clinical importance.

Perspectives

- Estrogen deficiency in postmenopausal women is linked with increased cardiovascular disease risk in association with augmented indexes of aortic wave reflection (AWRI) and elevated central blood pressure. We assessed whether estrogen deficiency in physically active women with functional hypothalamic amenorrhea (ExFHA) is also associated with increased AWRI or augmented central blood pressure.
- Compared with eumenorrheic physically active women, ExFHA women do not demonstrate increased AWRI and central blood pressure, rather they demonstrate lower values, yet they also present with higher vascular resistance. An acute bout of exercise, a known nitric oxide stimulus, did not alter AWRI in ExFHA. In contrast, vascular resistance and central blood pressure were lowered in ExFHA, with values remaining higher and lower, respectively, than in eumenorrheic women.
- Thus, functional arterial compliance and central blood pressure appear to be well preserved in ExFHA, despite hypoestrogenemia. However, unaltered AWRI one-hour post-exercise in ExFHA suggests vascular responsiveness to exercise-induced nitric oxide may be impaired or mitigated, possibly due to augmented vasoconstrictor tone.
- Collectively, we report complex interplay between vasoconstrictor tone, blood pressure and arterial compliance in ExFHA, likely in response to the competing effects of hypoestrogenemia, exercise training, and energy deficiency. These findings have relevance in understanding the cardiovascular consequences of hypoestrogenemia during the premenopausal years in otherwise healthy women.

Authorship: EOD wrote the manuscript, collected, analysed and interpreted the data; JMG edited the manuscript; JSF and PJH interpreted the data and edited the manuscript.

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Figure 1. Associations between indices of arterial stiffness (augmentation index [Alx] and augmentation index normalised for heart rate [Alx75]) and cardiac output (A and C, respectively) and total peripheral resistance (B and D, respectively) in ExFHA women. These associations were not observed ($p>0.05$) in ExOv women.

Table 1: Demographics of the study groups.

	ExOv	ExFHA	P
Age (yrs)	24±1	23±1	0.574
Height (m)	1.66±0.01	1.67±0.02	0.403
Body weight (kg)	57.8±1.7	57.1±3.4	0.857
BMI (kg/m ²)	21.1±0.5	20.2±0.9	0.389
Body fat (%)	24.4±2.3	24.1±1.9	0.946
Fat free mass (kg)	40.1±1.8	41.4±2.4	0.891
VO ₂ peak (ml/kg/min)	47.4±2.0	44.7±1.6	0.314
Free T3 (pmol/L)	4.2±0.2	3.5±0.2	0.029
Estradiol (pmol/L)	147±18	91±15	0.041
Progesterone (nmol/L)	1.6±0.2	1.7±0.2	0.831
Testosterone (nmol/L)	1.9±0.2	2.3±0.2	0.239
SHBG (nmol/L)	56.0±3.0	53.3±5.3	0.698
FAI (au)	0.42±0.01	0.47±0.01	0.592
Amenorrhea (days)*	-	885±248	-

Mean ± SEM

* Range 123-2160 days

Au, arbitrary units; BMI, body mass index; FAI, free androgen index; SHBG, sex hormone binding globulin; T3, triiodothyronine; VO₂ peak, peak oxygen uptake.

Table 2. Hemodynamic measures at rest and one hour post-exercise.

	ExOv		ExFHA	
	PreEx	PostEx	PreEx	PostEx
HR (b/min)	55±2	68±3 [†]	48±3*	59±3 ^{*†}
SBP (mmHg)	101±2	100±2	91±3*	91±3*
DBP (mmHg)	58±2	54±2 [†]	55±2	52±1 [†]
MAP (mmHg)	72±2	70±2 [†]	67±2*	65±2 [†]
PP (mmHg)	44±1	46±2 [†]	36±2*	40±2 ^{*†}
SV (mL/beat)	80±2	80±3	76±3	78±2
SVI (mL/beat/m ²)	49±1	49±2	46±2	47±2
CO (L/min)	4.14±0.13	5.02±0.21 [†]	3.40±0.18*	3.86±0.19 ^{*†}
CI (L/min/m ²)	2.55±0.11	3.08±0.13 [†]	2.08±0.09*	2.37±0.11 ^{*†}
TPR (dynes-sec/cm ⁵)	1406±54	1130±57 [†]	1614±84*	1375±77 ^{*†}
TPC (units)	58±2	73±4 [†]	51±3*	60±3 ^{*†}

Mean ± SEM

* versus ExOv within condition, P<0.05

† pre- versus post-exercise within groups, P<0.05

Table 3. Central blood pressure and indices of arterial stiffness at rest and one hour post-exercise.

	ExOv		ExFHA	
	PreEx	PostEx	PreEx	PostEx
SBPc (mmHg)	88±2	83±2†	80±2*	77±2*†
DBPc (mmHg)	59±2	56±2	56±2	52±2†
MAPc (mmHg)	72±2	68±2†	67±2*	63±2†
PPc (mmHg)	29±1	27±1	23±2*	25±1
AIx (%)	9.7±3.8	-2.3±3.5†	3.0±3.0	-0.2±3.6
AIx75 (%)	-0.6±3.3	-6.5±3.0†	-10.6±2.8*	-10.4±2.3
AP (mmHg)	3.1±1.2	-0.5±0.9†	0.6±0.7	-0.2±0.9

Values are mean±SEM

* versus ExOv within condition, $p<0.05$

† pre- versus post-exercise, within groups, $p<0.05$

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