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Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training

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Nyberg M, Seidelin K, Andersen TR, Overby NN, Hellsten Y, Bangsbo J. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise training. *Am J Physiol Regul Integr Comp Physiol* 306: R510–R517, 2014. First published January 17, 2014; doi:10.1152/ajpregu.00539.2013.—Menopause is associated with an accelerated decline in vascular function; however, whether this is an effect of age and/or menopause and how exercise training may affect this decline remains unclear. We examined a range of molecular measures related to vascular function in matched premenopausal and postmenopausal women before and after 12 wk of exercise training. Thirteen premenopausal and 10 recently postmenopausal [1.6 ± 0.3 (means ± SE) years after final menstrual period] women only separated by 3 yr (48 ± 1 vs. 51 ± 1 yr) were included. Before training, diastolic blood pressure, soluble intercellular adhesion molecule-1 (sICAM-1), and skeletal muscle expression of thromboxane A synthase were higher in the postmenopausal women compared with the premenopausal women, all indicative of impaired vascular function. In both groups, exercise training lowered diastolic blood pressure, the levels of sICAM-1, soluble vascular adhesion molecule-1 (sVCAM-1), as well as plasma and skeletal muscle endothelin-1. The vasodilator prostacyclin tended ($P = 0.061$) to be higher in plasma with training in the postmenopausal women only. These findings demonstrate that already within the first years after menopause, several biomarkers of vascular function are adversely altered, indicating that these biomarker changes are more related to hormonal changes than aging. Exercise training appears to have a positive impact on vascular function, as indicated by a marked improvement in the biomarker profile, in both premenopausal and postmenopausal women.

estrogen; soluble intercellular adhesion molecule-1; endothelin-1; prostanoids

VASCULAR DYSFUNCTION IS A condition that entails an imbalance between vasodilator and vasoconstrictor substances and a proinflammatory state. This alteration of the vasculature plays a crucial role in triggering and development of cardiovascular disease. Aging is associated with a decline in vascular function but appears to be delayed in premenopausal women compared with men (6). On the other hand, the decline in vascular function in women is accelerated during the postmenopausal period (6) and the time around menopause is also accompanied by additional unfavorable levels of several cardiovascular risk factors (2, 15, 22). However, to what extent a higher cardiovascular risk and a decline in vascular function in postmenopausal compared with premenopausal women is a function of aging and/or a consequence of menopause and its associated loss of estrogen remain unresolved (4). This lack of differentiation is, in part, related to the fact that most studies have included premenopausal and postmenopausal women that were separated by more than 15 yr. Trying to differentiate between the relative contributions from two concurrent and intimately linked processes is methodologically challenging; however, one approach is by investigating women of similar age (<5 yr) but with different menopausal status, as significant age-related vascular changes are likely to be limited within this short time frame.

Vascular dysfunction is associated with a proinflammatory and prothrombotic phenotype of the endothelium (16). Menopause and ovariectomy have been shown to cause low-grade systemic inflammation (1), which may contribute to atherosclerosis by a variety of mechanisms, depending on the stage of the disease (31). Circulating markers of systemic inflammation, including soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM) have been shown to be associated with atherosclerosis and cardiovascular events (21, 28, 41), but there is little information on how these biomarkers are affected by menopause and by exercise training.

Estrogen promotes formation of the vasodilator substance prostacyclin (24, 40), and this interaction may explain why this system is adversely affected after menopause (12, 37). Because of the very short half-life of prostacyclin (2–3 min), measurement of the more stable metabolite 6-keto $PGF_{1\alpha}$ can be used to quantify prostacyclin levels. The enzyme cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandin H_2 from which the two vasodilators prostacyclin (via PGI_2 synthase) and prostaglandin E_2 (via PGE synthase) and the vasoconstrictor thromboxane A_2 (via TBXA synthase) are derived. COX-1 and COX-2 isoforms are enzymatically active in skeletal muscle and may contribute to prostanoid formation (38).

Another compound important for vascular function is endothelin-1 (ET-1), which is produced mainly in the endothelium and plays an important role in the pathogenesis of vascular dysfunction associated with aging (19). Estrogen modulates the effects of ET-1 in the cardiovascular system (35), probably through its inhibitory effect of ET-1 formation (19). To what extent the prostanoid and ET-1 systems are affected in recent postmenopausal women remain unclear.

Estrogen modulates numerous molecular pathways that improve vascular function at physiological levels and to some extent when administered as hormone therapy (24). Nevertheless, clinical studies have questioned the protective value of hormone replacement therapy, as these studies indicate that such an intervention may increase cardiovascular disease risk

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and events in postmenopausal women (9, 32). Although early initiation of estrogen replacement has been shown to produce more favorable results (29, 33), the need for additional approaches to decrease the risk of cardiovascular disease remains apparent. Physical activity induces many of the same health-related effects as estrogen (24, 26), and physical activity could, therefore, potentially be used in the prevention and treatment of menopause-associated changes in the cardiovascular system.

Numerous methodologies have been developed to assess vascular function; however, since this is a multifaceted condition, the best methodology is still under debate (7). One validated approach to investigate vascular function is to measure biomarkers of vasoactive systems and inflammation (7). Therefore, our aim was to determine differences in biomarkers of vascular dysfunction in premenopausal and recent postmenopausal women of similar age (<5 yr) and to examine to what extent exercise training affects these variables. We hypothesized that recent postmenopausal women would display unfavorable levels of vasoactive and proinflammatory and anti-inflammatory biomarkers compared with premenopausal women and that exercise training would reverse the adverse change in these levels.

METHODS

Subjects

Thirteen middle-aged premenopausal [48 ± 1 (mean ± SE) years, maximal oxygen uptake ($\dot{V}O_{2\,max}$) 34.6 ± 1.9 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$] and 10 middle-aged postmenopausal (51 ± 1 years, $\dot{V}O_{2\,max}$ 31.9 ± 1.6 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$) women participated in the study (Table 1). The premenopausal women were all experiencing regular menstrual cycles and were not taking oral contraceptives, whereas the postmenopausal women had not experienced a menstrual cycle during the previous 12 mo but were less than 3 years past their final menstrual period (1.6 ± 0.3 years). None of the postmenopausal women were in hormone replacement therapy. Menopausal status was verified by measurements of hypothalamic and reproductive hormones (Table 1). All subjects were habitually inactive (less than 1 h of moderate-intensity exercise per week), had normal resting ECG, were nonsmokers, and none of the subjects in either group had been diagnosed with cardiovascular disease, renal dysfunction, insulin resistance, diabetes, or hypercholesterolemia. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg communities Region H (H-1–2012-150) and conducted in accordance with the guidelines of the Declaration of Helsinki. The subjects were informed of any risks and discomforts associated with the experiments before giving their written, informed consent to participate in the study.

Experimental Design

Two experimental days were performed before and after a 12-wk training period where the subjects participated in supervised exercise training. Progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations were determined during the first days of menses in the premenopausal women. Similarly, as lipoprotein levels change over the menstrual cycle, lipoprotein levels in the premenopausal women were determined during the first days of menses due to a reduced variability in the levels during this period and because this phase can be more reliably identified than others (23). Whole blood used for measuring lipoproteins was collected in a nonfasted state (17).

Exercise Training

Exercise training was performed as floorball, which is a team sport like hockey, but played indoors with plastic sticks (http://www.floorball.org). Each training session entailed ~30 min of technical and tactical floorball exercises, including warm up, and 30 min of game play with 4–6-min intervals (>85% of maximal heart rate) separated by 1–3 min of recovery. Training compliance and intensity are presented in Table 2. The subjects performed the training twice a week. The subjects wore a heart rate monitor (TEAM2 Wearlink+, Polar, Kempele, Finland) during each training session.

Table 1. *Baseline characteristics before and after 12 wk of exercise training*

	Premenopausal		Postmenopausal		
	Before	After	Before	After	
Variable	(*week 0*)	(*week 12*)	(*week 0*)	(*week 12*)	Overall Effect
Subjects	13		10		
Age, yr	48 ± 1		51 ± 1#		
Height, m	1.71 ± 0.02		1.69 ± 0.02		
Weight, kg	67.2 ± 3.7	67.9 ± 3.6	70.8 ± 3.5	71.3 ± 3.9	
Body mass index, kg/m^2	22.9 ± 0.8	23.2 ± 0.9	24.7 ± 1.0	25.0 ± 1.2	
Estradiol, nmol/l	0.41 ± 0.14		0.11 ± 0.05##		
Progesterone, nmol/l	1.3 ± 0.2		0.9 ± 0.2		
FSH, IU/l	13.0 ± 2.3		70.5 ± 9.8###		
LH, IU/l	8.4 ± 1.5		43.2 ± 4.6###		
Testosterone, nmol/l	0.75 ± 0.10		0.79 ± 0.23		
Erythrocytes, vol. fr.	0.39 ± 0.01	0.38 ± 0.01	0.40 ± 0.01	0.39 ± 0.00*	$P = 0{,}014$
$\dot{V}O_{2max}$, ml/min	2282 ± 104	2376 ± 122*	2236 ± 118	2355 ± 117**	$P < 0{,}001$
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	34.6 ± 1.9	36.4 ± 2.2	31.9 ± 1.6	33.5 ± 1.8*	$P = 0{,}009$
Body fat, %	32.4 ± 2.3	31.7 ± 2.2	39.2 ± 1.8#	37.4 ± 2.0**	$P < 0{,}001$
Lean body mass, kg	42.9 ± 1.5	43.7 ± 1.4*	41.1 ± 1.6	42.5 ± 1.8***	$P < 0{,}001$
Lean leg mass, kg	15.77 ± 0.55	16.24 ± 0.58**	14.85 ± 0.56	15.22 ± 0.59*	$P < 0{,}001$
CRP, mg/l	1.2 ± 0.1	1.1 ± 0.1	2.6 ± 1.1	1.9 ± 0.5	
Total cholesterol, mM	4.8 ± 0.2	4.9 ± 0.2	5.2 ± 0.4	5.2 ± 0.3	
HDL cholesterol, mmol/l	1.67 ± 0.10	1.73 ± 0.09	2.11 ± 0.22#	2.25 ± 0.18*#	$P = 0{,}018$
LDL cholesterol, mmol/l	2.8 ± 0.1	2.8 ± 0.2	2.8 ± 0.2	2.7 ± 0.3	

Values are expressed as means ± SE. FSH, follicle-stimulating hormone; LH, leutinizing hormone; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Significantly different from before training: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Significantly different from premenopausal: #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$.

Table 2. *Training compliance and intensity*

	Training Sessions		Average Time per Session, min	Total Training Time (% of HR_{max}), %						
	Per Week	Total		<61%	61–70%	71–80%	81–85%	86–90%	91–95%	96–100%
Premenopausal	1.7 ± 0.1	20.1 ± 1.0	53.8 ± 0.9	25.8 ± 4.1	20.4 ± 1.0	19.8 ± 1.9	11.2 ± 0.7	12.2 ± 1.1	9.0 ± 2.1	1.6 ± 0.5
Postmenopausal	1.8 ± 0.1	22.0 ± 0.7	55.6 ± 1.1	31.5 ± 6.3	19.7 ± 2.2	19.5 ± 1.6	9.7 ± 1.8	8.6 ± 1.7	7.3 ± 2.3	3.8 ± 1.7

Values are expressed as means ± SE. HR, heart rate.

Experimental Days

All experimental testing performed on *days 1* and *2* in the premenopausal women were performed in the midfollicular phase of their ovarian cycle.

Day 1. Subjects refrained from exercise for 24 h before reporting to the laboratory between 0800 and 1000 AM after an overnight fast. After 10 min of rest in a supine position, a catheter (20 gauge, 32 mm) was inserted into the antecubital vein for collection of blood. To avoid interference due to blood stasis, blood samples were drawn 15 min after venous cannulation. Body composition was then determined from whole-body dual-energy X-ray absorptiometry scanning (Prodigy, GE Medical Systems, Milwaukee, WI), and the subjects rested for at least 15 min in supine position before blood pressure was measured seven consecutive times by an automatic upper arm blood pressure monitor (M7, OMRON, Vernon Hills, IL). After an additional ~20 min of rest, a muscle biopsy was obtained from musculus vastus lateralis using the percutaneous needle biopsy technique (3).

Day 2. Subjects reported to the laboratory to perform an incremental running exercise test in which $\dot{V}O_{2\,max}$ was determined (Oxycon Pro, Intramedic, Denmark; Table 1). Briefly, the treadmill test protocol consisted of 2 × 4 min of walking (4 km/h and 6 km/h) interspersed with 2 min of rest. After the two submaximal bouts, an incremental test to exhaustion was performed starting with 2 min at 6 km/h. Hereafter, running speed was increased by 1 km/h until volitional fatigue.

Measurements and Analyses

Maximal heart rate was determined as the highest value obtained during the incremental running exercise test or any of the training sessions. Whole blood samples were analyzed at the clinical biochemical unit at the Copenhagen main hospital (Rigshospitalet) using an automatic analyzer using enzymatic kits (Modular P-Module) for total cholesterol, low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), erythrocyte volume fraction by using cumulated resistance (Sysmex XE-2100), C-reactive protein by using turbidimetric immunoassay (Modular P-Module), estradiol (Modular P-Module), progesterone, and testosterone (Modular E-Module) by using competitive electrochemiluminescence immunoassay and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by using sandwich electrochemiluminescence immunoassay (Modular E-Module).

Blood samples for collection of plasma were drawn into tubes containing EDTA as anticoagulant. Blood was then immediately centrifuged for 15 min at 1,000 *g*, and plasma was then stored at −20°C for later analysis. Plasma concentrations of ET-1 (Orion L Microplate Luminometer, 300–600 nm; Titertek-Berthold, Huntsville, AL), sVCAM-1, and sICAM-1 (Emax precision microplate reader; Molecular Devices, Sunnyvale, CA) were measured using an immunoassay (ELISA; R&D Systems, Minneapolis, MN) and the stable metabolite of PGI_2, 6-keto prostaglandin $F_{1\alpha}$, was measured (Emax precision microplate reader; Molecular Devices) using an enzyme immunoassay kit (Cayman Chemical, Ann Harbor, MI).

Quantification of Protein Expression

In the muscle samples, protein expression was determined by Western blot analysis, as previously described (25). Briefly, ~3 mg dry weight of the biopsy was homogenized in homogenization buffer, and the protein concentration of the lysate samples was determined (assayed in triplicate; only a coefficient of variation of less than 5% was accepted). Lysate proteins were separated using 10% SDS gels (Bio-Rad Laboratories, Hercules, CA) and transferred to PVDF membranes (Immobilion Transfer Membrane; Millipore, Billerica, MA). The membranes were incubated with primary polyclonal antibodies against ET-1 (1:1,000; Abcam, Cambridge, UK), cycloxygenase-1 (1:500, COX-1; Abcam), cyclooxygenase-2 (1:500, COX-2; Abcam), prostacyclin synthase (1:200, PGI_2 synthase; Santa Cruz Biotechnology, Santa Cruz, CA) and thromboxane A synthase (1:500; TBXA synthase). Secondary antibody horseradish-peroxidase-conjugated goat anti-rabbit (COX-1, COX-2, PGI_2 synthase, and TBXA synthase) and anti-mouse (ET-1) was used for detection. The protein content was expressed in arbitrary units relative to mixed human skeletal muscle standard samples run on each gel. Equal amounts of total protein were loaded for each sample, and samples from each group were distributed evenly across the gel. All samples were run simultaneously, and the sample from before training was placed adjacent to the sample after training for each subject.

Statistical Analysis

Differences in age, height, estradiol, progesterone, LH, FSH, and testosterone concentrations and training compliance and intensity were assessed with a Student's *t*-test. Specific hypothesis testing was performed with two-way repeated-measures ANOVA. Following a significant *F* test, pair-wise differences were identified using the Tukey's honestly significant difference post hoc procedure. SigmaPlot 11.0 (Systat Software, San Jose, CA) was used for all analyses. The significance level was set at $P < 0.05$, and data are expressed as means ± SE unless otherwise indicated.

RESULTS

Maximal Oxygen Uptake and Blood Pressure

There was no difference in $\dot{V}O_{2\,max}$ between premenopausal and postmenopausal women (2,282 ± 104 vs. 2,236 ± 118 ml/min) before the training period (Table 1). $\dot{V}O_{2\,max}$ was 4.6 ± 1.8% higher ($P < 0.05$) in premenopausal and 5.5 ± 1.6% higher ($P < 0.01$) in postmenopausal women after exercise training compared with before the training period.

Systolic blood pressure was similar between premenopausal and postmenopausal women and was unchanged with exercise training (Fig. 1*A*). Diastolic blood pressure was higher ($P < 0.05$) in postmenopausal women compared with premenopausal women (79 ± 3 vs. 71 ± 3 mmHg) before training (Fig. 1*B*). Training lowered diastolic blood pressure by 2.8 ± 1.3 ($P < 0.05$) and 3.9 ± 1.7 mmHg ($P < 0.01$) in premenopausal and postmenopausal women, respectively (main effect; $P < 0.001$). Diastolic blood pressure tended ($P = 0.065$) to be higher in postmenopausal women compared with premenopausal women after training.

Body Composition

The percentage of fat was higher ($P < 0.05$) in postmenopausal women than in premenopausal women (39.2 ± 1.8 vs.

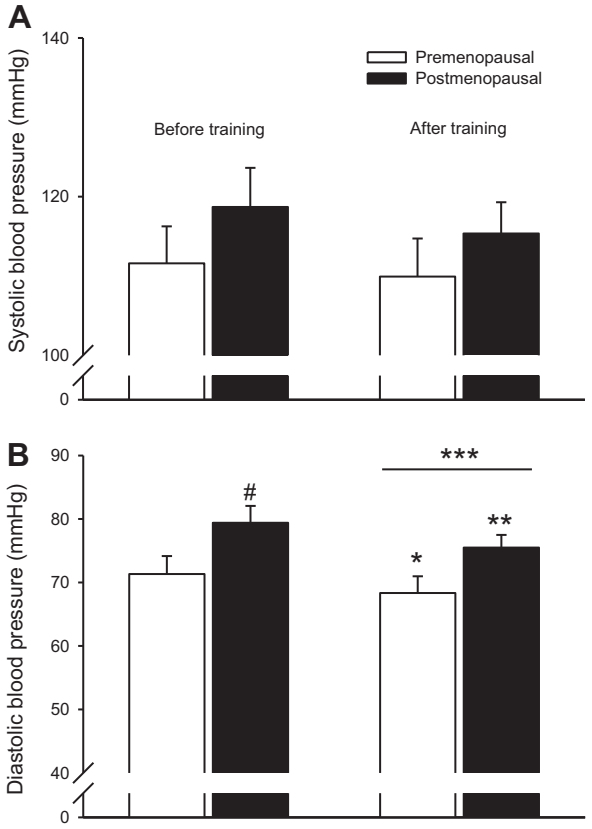

Fig. 1. Systolic (*A*) and diastolic (*B*) blood pressure measured at rest before and after 12 wk of training for premenopausal and postmenopausal women. Values are expressed as means ± SE. Significantly different from premenopausal, #$P < 0.05$. Significantly different from before training, *$P < 0.05$,**$P < 0.01$ (main effect of training, ***$P < 0.001$).

32.4 ± 2.3%) before training, but not after the training period due to a lowering ($P < 0.01$) of body fat in the postmenopausal women only (Table 1). There was no difference in lean body mass between premenopausal and postmenopausal women (42.9 ± 1.5 vs. 41.1 ± 1.6 kg) before training. Lean body mass increased by 0.80 ± 0.32 ($P < 0.05$) and 1.43 ± 0.35 kg ($P < 0.001$) in premenopausal and postmenopausal women, respectively.

sVCAM-1 and sICAM-1

There was no difference in sVCAM between premenopausal and postmenopausal women (674 ± 55 vs. 720 ± 54 ng/ml) before the training period (Fig. 2*A*). Training lowered the concentration of sVCAM in both premenopausal ($P < 0.001$) and postmenopausal ($P < 0.01$) women by 139 ± 44 and 115 ± 32 ng/ml, respectively (main effect; $P < 0.001$). The concentration of sICAM-1 was lower ($P < 0.05$) in premenopausal women than in postmenopausal (166 ± 6 vs. 208 ± 22 ng/ml) women before training (Fig. 2*B*). Training lowered the concentration of sICAM in both premenopausal ($P < 0.001$) and postmenopausal ($P < 0.001$) women by 44 ± 5 and 37 ± 12 ng/ml, respectively (main effect; $P < 0.001$), and the concentration of sICAM-1 was also higher in postmenopausal compared with premenopausal women after the training period.

Plasma and Skeletal Muscle Endothelin-1 Levels

The plasma (1.62 ± 0.25 vs. 1.47 ± 0.15 pg/ml) and skeletal muscle (1.52 ± 0.18 vs. 1.58 ± 0.20 AU) level of ET-1 was not different between the premenopausal and postmenopausal women before training (Fig. 3, *A* and *B*). Training lowered (main effect, $P < 0.01$) the plasma concentration of ET-1 in the premenopausal women by 0.66 ± 0.24 pg/ml and tended ($P = 0.072$) to lower (0.40 ± 0.13 pg/ml) the plasma concentration of ET-1 in the postmenopausal women and the level of skeletal muscle ET-1 (main effect, $P < 0.05$).

Plasma 6-Keto Prostaglandin $F_{1\alpha}$ and Skeletal Muscle Cyclooxygenase-1, Cyclooxygenase-2, Prostacyclin Synthase, and Thromboxane A Synthase

There was no difference in the level of plasma 6-keto $PGF_{1\alpha}$ (Fig. 4*A*) and skeletal muscle COX-1, COX-2, and PGI_2 synthase (Fig. 4*B*) between premenopausal and postmenopausal women before training, whereas the level of TBXA synthase was higher ($P < 0.05$) in the postmenopausal women. Plasma 6-keto $PGF_{1\alpha}$ tended ($P = 0.061$) to be higher after training in the postmenopausal women and training increased ($P < 0.05$) the skeletal muscle level of TBXA synthase in the premenopausal women (0.62 ± 0.11 vs. 1.09 ± 0.18 AU). There was no effect of training on the level of COX-1, COX-2, and PGI_2 synthase in skeletal muscle.

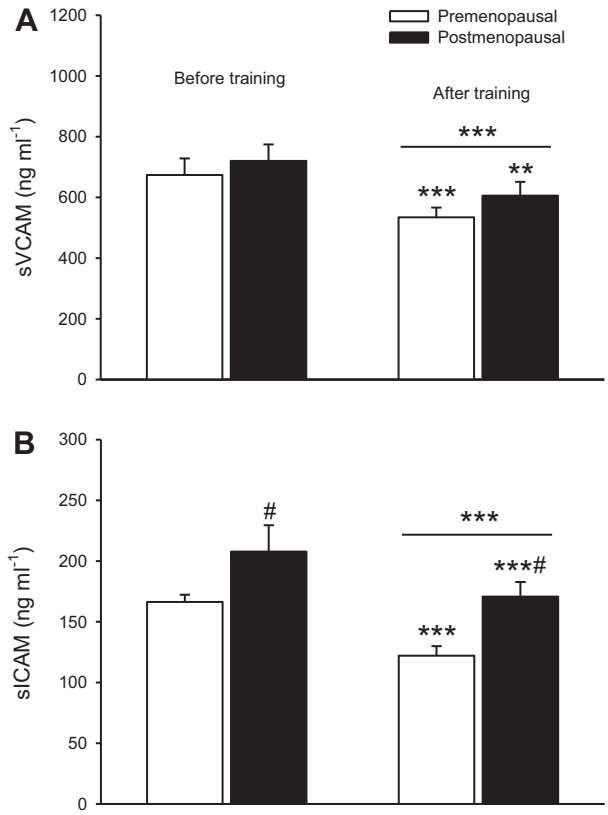

Fig. 2. Plasma soluble vascular adhesion molecule-1 (sVCAM-1; *A*) and soluble intercellular adhesion molecule-1 (sICAM-1; *B*) concentrations measured at rest before and after 12 wk of training for premenopausal and postmenopausal women. Values are expressed as means ± SE. Significantly different from before training, **$P < 0.01$, ***$P < 0.001$ (main effect of training, ***$P < 0.01$), significantly different from premenopausal, #$P < 0.05$.

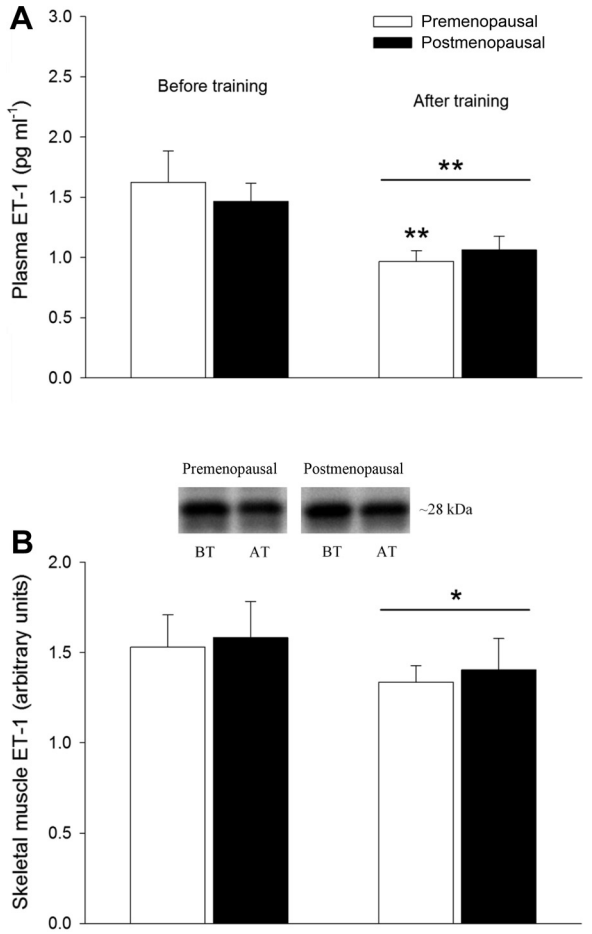

Fig. 3. Plasma endothelin-1 (ET-1) concentrations (*A*) and skeletal muscle ET-1 levels (*B*) measured at rest before and after 12 wk of training for premenopausal and postmenopausal women. Values are expressed as means ± SE. Significantly different from before training $*P < 0.05$, $**P < 0.01$ (main effect of training, $*P < 0.05$, $**P < 0.01$). BT: before training, AT: after training.

DISCUSSION

The primary findings of the present study were that recent postmenopausal women had higher levels of the vascular inflammatory marker sICAM-1, diastolic blood pressure, and expression of skeletal muscle TBXA synthase than premenopausal women of similar age. Furthermore, exercise training improved the level of numerous biomarkers associated with vascular function and cardiovascular risk in both groups of women.

ICAM-1 is an endothelial adhesion molecule that stimulates leukocyte adhesion and transmigration into the vascular subendothelial space, and this process, when unabated, is implicated in endothelial dysfunction, tissue injury, and ensuing atherosclerosis (30). ICAM-1 is preferentially expressed in endothelium overlying human atheroma (27), and circulating sICAM-1 is the result of cleavage of membrane-bound ICAM-1. In the present study, the concentration of sICAM-1 was found to be higher in the postmenopausal than in the premenopausal women. As sICAM-1 has been suggested to be an early biomarker of changes in the artery wall that accompany atherosclerosis (11), the current findings suggest that adverse menopause-induced changes in the integrity of the endothelium can be detected at an early stage following amenorrhea. Estrogen treatment has been shown to reduce the level of sICAM-1 in postmenopausal women (14), indicating that the loss of estrogen is one the mechanisms underlying the increase in sICAM-1 after menopause. The finding that the training period reduced the levels of sICAM-1 in the postmenopausal women to levels not significantly different from those of the premenopausal women before training suggest that exercise training mimics the effects of estrogen on vascular inflammation in recent postmenopausal women.

The level of the adhesion molecule sVCAM-1, which is also important in focal leukocyte accumulation in subendothelial regions of atheroma, was found to be similar between the two groups of women. sVCAM is associated with cardiovascular events among patients (8), indicating that sVCAM-1 only

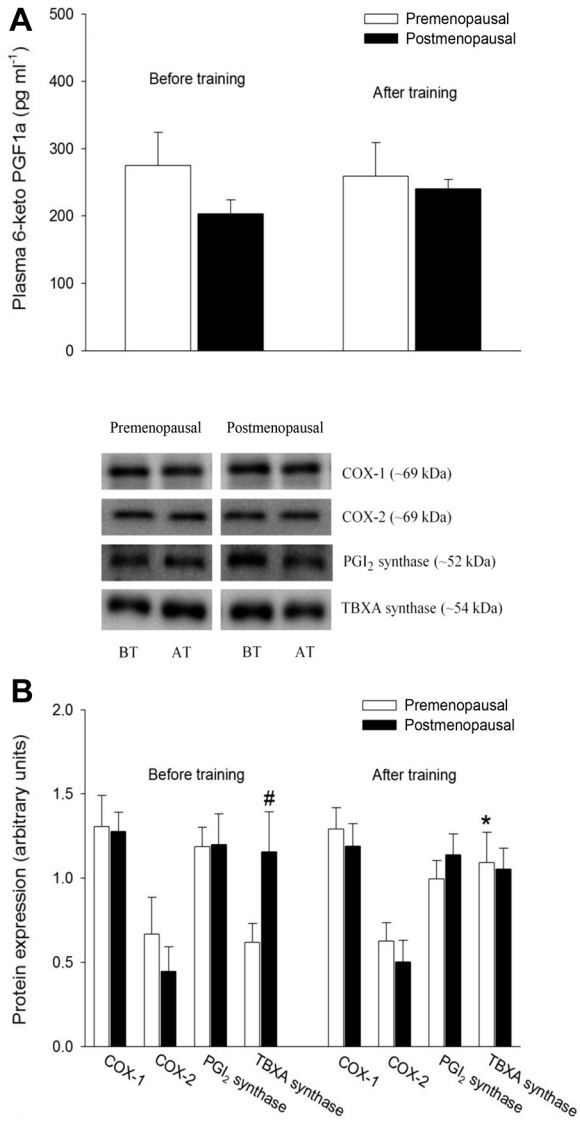

Fig. 4. Plasma 6-keto $PGF_{1\alpha}$ concentrations (*A*) and skeletal muscle cyclooxygenase (COX-1), COX-2, PGI_2 synthase and thromboxane A2 (TBXA) synthase levels (*B*) measured at rest before and after 12 wk of training for premenopausal and postmenopausal women. Values are expressed as means ± SE. Significantly different from premenopausal: $\#P < 0.05$. Significantly different from before training: $*P < 0.05$. BT, before training; AT, after training.

increases during more severe inflammation-induced alterations of the endothelium. Nevertheless, the reduction in sVCAM-1 in both groups of women after a period of exercise training is indicative of an improved function and integrity of the vasculature.

Diastolic blood pressure is strongly related to cardiovascular mortality (20). Despite a similar level of fitness and age, diastolic blood pressure was higher in the postmenopausal women before the training period. Blood pressure has consistently been reported to be higher in postmenopausal women compared with premenopausal women; however, this has been reported to be due to an increase in systolic blood pressure as diastolic blood pressure was found to be unchanged or lower (5). Reduction in arterial compliance, which is associated with aging, leads to a higher systolic blood pressure and lower diastolic blood pressure (13). Thus, the current finding that the postmenopausal women had higher diastolic blood pressure suggests that one of the early effects of estrogen depletion is an increase in vascular resistance that is not related to arterial stiffening.

ET-1 exerts potent vasomotor, proinflammatory, and proatherogenic effects on the vascular system. Given the role of estrogen in modulating ET-1-mediated vascular effects (19, 35), a potential role for ET-1 in the decline in endothelial function associated with menopause seems likely. As a consequence of the large mass of skeletal muscle, a large proportion of cardiac output is directed to this tissue, and alterations in skeletal muscle could, therefore, contribute significantly to the decline in vascular function in postmenopausal women. Accordingly, skeletal muscle levels of ET-1 have been found to be higher in lifelong sedentary older men who also displayed impaired endothelium-dependent vasodilation (25). However, in the current study, both plasma and skeletal muscle levels of ET-1 were found to be similar in the two groups of women, which suggest that a potential role for ET-1 in the progression of vascular dysfunction following menopause is related to mechanisms downstream from receptor binding, which is in congruence with findings demonstrating that plasma ET-1 concentrations do not correlate with the contribution of ET to baseline vascular tone (39).

In premenopausal women, the urinary excretion of prostacyclin has been found to be higher compared with postmenopausal women (12). As prostacyclin is a potent vasodilator and potent inhibitor of platelet aggregation, this effect of menopause could play an important role in the progression of atherosclerosis and endothelial dysfunction. Although this did not reach statistical significance, the level of the stable metabolite of prostacyclin, 6-keto $PGF_{1\alpha}$, was ~30% lower in the postmenopausal women compared with the premenopausal women. Furthermore, exercise training tended ($P = 0.061$) to increase the level of plasma 6-keto $PGF_{1\alpha}$ in the postmenopausal women only. As estrogen has been shown to upregulate the production of prostacyclin (34), the present findings suggest that estrogen deprivation reduces prostacyclin availability at an early stage following the menopause transition and that exercise training can be used as an intervention to increase these levels.

With aging, inhibition of COX reveals a vasoconstrictor role for prostanoids (36). In the present study, skeletal muscle COX-1 and COX-2 levels were similar in premenopausal and postmenopausal women. However, the skeletal muscle level of TBXA synthase was found to be higher in the postmenopausal women compared with the premenopausal women, indicating that thromboxane A_2 formation was elevated. As thromboxane A_2 is both a vasoconstrictor and a potent stimulator of platelet aggregation, and given the large mass of skeletal muscle, this observation indicates that an increased formation of thromboxane A_2 in skeletal muscle could be one of the mechanisms underlying vascular dysfunction. Future studies should attempt to clarify this possibility. Interestingly, TBXA synthase was higher after the training period in the premenopausal women only. This finding is in congruence with observations on older men (10) and indicates that an increased capacity to form thromboxane A_2 is needed to balance the upregulation of vasodilator systems also associated with exercise training when vascular function is not adversely affected by estrogen deficiency.

High-intensity exercise training has been shown to induce greater aerobic and cardiovascular adaptations than training performed at low and moderate levels in both healthy subjects (18) and patients diagnosed with cardiovascular disease (43). Within the present conditions, exercise training that included periods with high intensity improved the levels of several markers of vascular function and cardiovascular risk factors in both premenopausal and postmenopausal women. Notably, these cardiovascular adaptations resulted from ~2 training sessions per week across the 12-wk training intervention. These findings suggest that the current exercise training regime is an effective and time-conserving approach to improve vascular function and cardiovascular risk in middle-aged women.

When applying a commonly used approach to predict initial cardiovascular events in healthy subjects (42), the 10-year risk before the training period in the premenopausal and postmenopausal women was found to be 2.8 ± 0.9 and $3.1 \pm 0.5\%$, respectively. This risk assessment indicates that the postmenopausal women were not at a considerable higher risk of developing cardiovascular disease compared with the premenopausal women. Nevertheless, in the current study, we examined several biochemical markers of vascular function that are less commonly examined and that are not included in risk assessments. These measurements revealed that the postmenopausal women displayed unfavorable levels of several markers of vascular function and vascular health, indicating early adverse vascular changes.

Perspectives and Significance

The current study demonstrates that several biomarkers of vascular function are adversely affected in recent postmenopausal women compared with premenopausal women of similar age. This effect during early onset of menopause is likely to be important for cardiovascular health later in life, as vascular dysfunction precedes the development of cardiovascular disease. Therefore, the current findings pinpoint putative mechanisms underlying vascular dysfunction in recent postmenopausal women that may be important for the development of cardiovascular disease later in life, and these mechanisms should be the focus of future investigations. Exercise training has a positive impact on the vascular biomarker profile, and the use of physical activity to offset menopause-induced changes in other measures of vascular function should also be explored.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.N., T.R.A., Y.H., and J.B. conception and design of research; M.N., K.S., T.R.A., N.N.O., and J.B. performed experiments; M.N., K.S., T.R.A., and N.N.O. analyzed data; M.N., Y.H., and J.B. interpreted results of experiments; M.N. prepared figures; M.N. drafted manuscript; M.N., Y.H., and J.B. edited and revised manuscript; M.N., K.S., T.R.A., N.N.O., Y.H., and J.B. approved final version of manuscript.

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