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ORIGINAL ARTICLE

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Calf blood flow is augmented in habitually aerobically trained versus untrained postmenopausal women in association with favorable modulation of vasculo-metabolic interactions

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Abstract

Introduction: Menopause is associated with vascular dysfunction and increased risk of developing metabolic syndrome. Associations between vascular and metabolic health, and interactions with aerobic exercise training, are unknown in postmenopausal women (PMW).

Methods: In habitually aerobically trained PMW (PMWtr; $n = 10$; 57 ± 1 years; 40 ± 1 mL/kg/min), strain-gauge plethysmography was used to compare resting and peak calf blood flow (CBFr and CBFpk, respectively) and vascular resistance (CVRr; CVRpk) versus untrained PMW (PMWun; $n = 13$; 56 ± 1 years; 29 ± 1 mL/kg/min) and premenopausal women (PreM; $n = 14$; 26 ± 1 years; 40 ± 1 mL/kg/min). Vascular measures were taken before and 1 hour after 45 minutes of aerobic exercise (60% $\dot{V}O_{2peak}$), a known nitric oxide stimulus. Blood analyses included low- (LDLc) and high-density lipoprotein cholesterol (HDLc), insulin, and glucose.

Results: Pre-exercise, CBFr and CVRr did not differ ($p > 0.05$) between PMW groups, nor between PreM and PMWtr. CBFpk was highest ($p < 0.05$) and CVRpk was lowest ($p < 0.05$) in PMWtr. Blood markers were similar ($p > 0.05$) in PMW groups. However, in PMWtr, CBFpk was associated inversely ($p < 0.05$) with insulin ($r = -0.725$). Conversely, in PMWun, CBFpk correlated ($p < 0.05$) inversely with glucose ($r = -0.717$), positively with HDLc ($r = 0.633$), and CVRpk positively ($p < 0.05$) with LDLc ($r = 0.568$). Post-exercise, CBF increased and CVR decreased ($p < 0.05$) in all groups, yet CBFpk remained higher and CVRpk lower ($p < 0.05$) in PMWtr.

Conclusion: In untrained PMW, peak CBF is associated inversely with circulating pro-atherogenic lipids and glucose. In contrast, peak CBF is associated

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inversely with insulin levels only in trained PMW. Habitual aerobic exercise may favorably modulate vasculo-metabolic interactions in PMW.

KEYWORDS
endothelial function, estrogen deficiency, exercise, lipids, metabolic

1 | INTRODUCTION

Estrogen deficiency due to menopause is associated with increased risk of cardiovascular disease (CVD), including elevated pro-atherogenic lipids, central adiposity, increased arterial blood pressure, and unfavorably altered metabolic milieu.[1] Consequently, menopause is also associated with increased risk of metabolic syndrome, comprised of dyslipidemia, hyperinsulinemia, and obesity.[1] Independently, components of the metabolic syndrome are linked with vascular dysfunction in association with impaired production of nitric oxide (NO), a potent vasodilator.[2] Estrogen deficiency is also independently associated with impaired vascular function.[3,4] Estrogen increases the production and bioavailability of endothelium-derived NO via the PI3K/Akt pathway.[5] Conversely, estrogen deficiency decreases NO production[3] resulting in endothelial dysfunction, a key early step in the development and progression of atherosclerosis.[6] Thus, estrogen deficiency contributes importantly to cardiovascular disease risk in women.

Aerobic exercise training elicits beneficial effects on cardiovascular health, including vascular health.[7] In the vasculature, exercise-mediated increases in shear stress stimulate the production of endothelium-derived NO, also through the PI3K/Akt pathway.[8] Repeated exposure of the vasculature to increases in shear stress, such as with regular exercise, is associated with augmented vascular function[8]; however, sex differences are reported. In middle-aged and older men, aerobic exercise training has been consistently demonstrated to attenuate the age-associated decline in both macrovascular (e.g., conduit vessel) and microvascular (e.g., resistance vessel) endothelial function.[9–13] In contrast, studies in aerobically trained PMW are equivocal, with some[3,13,14] but not all[15,16] reporting no benefit of exercise training on macrovascular or microvascular endothelial function. Mechanisms of action are unclear, but a loss of vascular adaptations to exercise training due to estrogen deficiency[3] and aging *per se*[17] are implicated. Understanding the mechanisms of peripheral vasomodulation is of critical importance for PMW due to their increased risk of cardiovascular disease and metabolic syndrome.[1] Further, given the recommendation of regular exercise training for the management of cardiovascular and metabolic health in women after menopause,[18] understanding the modulatory effects of exercise training on mechanisms of vasomodulation is also of significance.

Accordingly, we examined interactions between calf blood flow (CBF), using venous-occlusion strain-gauge plethysmography, and circulating metabolic biomarkers in healthy untrained and habitually aerobically trained PMW. Untrained premenopausal women (PreM) were also studied to provide a healthy reference comparator group. To investigate the vascular response to an acute bout of dynamic exercise, a known NO stimulus, resting, and peak ischemic CBF was assessed before and 1 h after exercise. Serum levels of nitrite, insulin, glucose, and lipids, were also determined. We hypothesized that habitually aerobically trained PMW would demonstrate augmented basal CBF in association with more favorable levels of circulating metabolic biomarkers compared with untrained PMW. It was further postulated that after exercise, CBF would be augmented in both PMW groups in association with increased nitrite levels, but that increases in CBF would be greater in trained PMW in association with higher nitrite levels. Due to the known effects of aging on cardiovascular health,[13] we anticipated that PreM would demonstrate higher CBF and a more favorable metabolic biomarker profile than PMW.

2 | METHODS

2.1 | Participants

Participants were recruited from Loughborough University and the local community via advertisement. For PMW, eligibility criteria included: (i) aged 50–60 years; (ii) self-reported amenorrhea >12 months; (iii) no hormone replacement therapy use >6 months; and (iv) participation in structured exercise <2 days/week for the last 5 years (e.g. untrained) OR >3 days/week for the last 5 years (e.g. trained). For PreM, eligibility criteria included: (i) aged 18–35 years; (ii) regular menstrual cycles (10–13 per year) OR using combined oral contraceptives; (iii) participation in structured exercise <2 days/week. Exclusion criteria for all participants included: (i) current illness; (ii) known chronic diseases; (iii) hypertension (>140/90 mmHg); (iv) smoker; (v) BMI >30 kg.m^2; (vi) not weight stable for last 3 months and/or currently dieting; (vii) inability to complete the required exercise demands; and (viii) taking medications and/or dietary supplements. Additional exclusion criteria for PreM were: (i) pregnant;

(ii) breastfeeding; (iii) taking any form of continuous hormonal contraceptive. The research protocols conform with the ethical guidelines of the 1975 Declaration of Helsinki and received approval from the Loughborough University Human Participants Ethics Board. All subjects provided voluntary written informed consent prior to study entry.

2.2 | Experimental design

Participants were recruited consecutively over 3 years to participate in a larger cross-sectional study examining the cardiovascular consequences of estrogen deficiency in postmenopausal women. Forty women ($n = 26$ PMW and $n = 14$ PreM) 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, blood biomarkers, heart rate, and blood pressure for many of the PMW and PreM.[19] However, in the current study, for the first time we report CBF, associations between CBF and circulating metabolic biomarkers, and central hemodynamics in 37 of the 40 women.

2.3 | Study groupings

Three groups were studied: (i) habitually aerobically exercise trained postmenopausal women (PMWtr; $n = 10$), (ii) untrained postmenopausal women (PMWun; $n = 13$), and (iii) untrained premenopausal women (PreM; $n = 14$). PMWtr performed regular aerobic exercise involving the lower limbs ($n = 8$ runners, $n = 1$ netballer; $n = 1$ swimmer+power walking). Objective criterion measures of exercising status in PMW included a peak aerobic capacity of <35 mL/kg/min for untrained status and ≥ 35 mL/kg/min for exercise-trained status.[20]

2.4 | Experimental protocol

Measurements were obtained in a quiet, ambient room (22–24°C) and the supine position. Participants attended the laboratory in the morning after an 8 h fast and also abstained from caffeine, alcohol, and exercise for 24 h. After 20 min of quiet supine rest, baseline blood samples and measures of blood pressure (BP), heart rate (HR), and calf blood flow (CBF) were completed. Ultrasound measures of cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR) were also determined. Participants then completed 45 min of treadmill exercise at a HR corresponding to 60% of $\dot{V}O_{2peak}$.[19] To avoid sensations of hunger or hypoglycemia post-exercise, subjects consumed a standard light low-fat lunch immediately after exercise. All baseline measures were repeated, beginning 60 minutes after exercise. To control for the influence of the fluctuations in ovarian hormones due to the menstrual cycle, regularly menstruating PreM were tested during the early follicular (low estrogen and low progesterone) phase of the menstrual cycle (days 2–8). PreM using oral contraceptives were assessed during the 7 days pill-free/placebo period.

2.5 | Peak oxygen uptake

On a separate day at least 48 hours prior to the main experimentation, a graded exercise test was completed to determine peak oxygen uptake ($\dot{V}O_{2peak}$). Participants ran at a self-selected speed on a motorized treadmill (ExciteMed, TechnoGym, Cesena, Italy) with the incline increased by 1% every 2 minutes until minute 8, after which the incline was increased every minute until volitional exhaustion. Expired gas samples were analyzed continuously on a breath-by-breath basis using an online calibrated metabolic cart (Metalyzer 3B, Cortex, Leipzig, Germany). HR was continuously recorded using a short-range telemetry device (Polar T31, Kempele, Finland).

2.6 | Hemodynamic measures

Brachial systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP;1/3 pulse pressure + DBP) and HR were recorded in triplicate, 1 min apart, on the left upper arm using an automated device (Omron M6 AC ME). The average of the 3 measures was used in the statistical analyses.

Echocardiographic measures of the aortic root annulus during mid-systole were performed using continuous wave Doppler (2.0–8.0 MHz) in the right parasternal long-axis view. The maximum instantaneous aortic flow velocity was measured in the ascending aorta using continuous wave Doppler (2.0 MHz) directed through the suprasternal window.[21] Stroke volume (SV) was calculated from the product of the cross-sectional area of the aortic annulus and the mean time velocity integral.[21] Cardiac output (CO) was calculated from the product of SV and HR. Total peripheral resistance (TPR; 80*[MAP/CO]) and total peripheral conductance (TPC; [CO/MAP]*1000) were calculated.

2.7 | Calf blood flow (CBF)

CBF was assessed using venous-occlusion strain-gauge plethysmography as previously described.[22] In brief, the subject lay in a supine position with the calf slightly

elevated above the heart level. The calf was isolated by placing pneumatic cuffs (Hokanson) at the ankle and above the knee. An indium-gallium strain gauge (Vasculab SPG16 Medasonics) was placed around the widest girth of the calf. At the ankle, an exclusion cuff was inflated to 200 mmHg for 1 min, after which a 'collecting' cuff placed around the thigh was rapidly inflated (~50 mmHg) and deflated using 7 s intervals for 1 min. Peak-ischemic measures were acquired immediately following 5 min of occlusion at 200 mmHg. Data were captured and analyzed using LabChart Pro (v8, ADInstruments). Data from the slope of the time-leg volume curve was used to determine blood flow (mL/100 mL/min). Vascular resistance (MAP/CBF; arbitrary units [U]) were calculated.

2.8 | Blood biomarkers

Blood samples were collected from the antecubital vein using standard venepuncture techniques. After centrifuging, serum and plasma samples were stored at −80°C until analysis. Samples were analyzed for concentrations of 17β-estradiol, progesterone, testosterone, sex-hormone binding globulin, blood lipids, glucose, insulin, triiodothyronine, and thyroxine. Serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), and low-density lipoprotein cholesterol (LDLc) were analyzed using enzymatic calorimetric methods (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). Plasma insulin was assessed using an enzyme-linked immunosorbent assay (ELISA; Mercodia). Plasma nitrite was determined using a Sievers Nitric Oxide Analyzer gas-phase chemiluminescence nitric oxide analyzer (Analytix Ltd). Intra-assay coefficient of variation (%) values for plasma glucose, TC, LDLc, HDLc, and TG were 0.8, 1.5, 1.3, 1.2, and 1.1, respectively. Intra-assay coefficient of variation for insulin was 8.04%. Serum estradiol, progesterone, testosterone, triiodothyronine, thyroxine, and sex-hormone binding globulin were analyzed using direct chemiluminescent methods (Siemens Advia Centaur XPT) at the core laboratory at Leicester Hospital).

2.9 | Statistical analysis

Data were analyzed using IBM SPSS Statistics Software for Windows version 25 (IBM Corporation). Data were checked for normality using the Shapiro- Wilk test. Plasma nitrite was identified as having non-normal distribution and was log10 transformed for statistical analysis. Baseline subject characteristics were analyzed using one-way analysis of variance (ANOVA). Significant differences were detected using Bonferroni-adjusted post hoc tests. In PreM, student's t-tests were performed for paired observations (pre- versus post-exercise). For hemodynamic and vascular variables of interest, planned comparisons using Bonferroni-adjusted independent samples t-tests were used to compare PreM vs PMWun and PreM vs PMWtr both before and after exercise. The independent and combined effects of training status and acute exercise on vascular and hemodynamic responses in PMW were assessed using two-factor repeated-measures ANOVAs, with one within factor (time) and one between factor (group). Pearson-product correlational analyses were performed to identify significant linear associations between vascular function and variables of interest. Statistical significance was accepted at the $p < 0.05$ level. Unless otherwise stated, data are presented as mean ± SEM.

3 | RESULTS

3.1 | Subject characteristics

Groups did not differ ($p > 0.05$) in height, body mass, BMI, SBP, DBP, MAP, HR, and calf circumference (Table 1). PMWun and PMWtr were similar in age ($p > 0.05$) and older than PreM ($p < 0.05$). PMWun had lower $\dot{V}O2peak$ than PreM ($p < 0.05$) and PMWtr ($p < 0.05$). $\dot{V}O2peak$ did not differ ($p > 0.05$) between PreM and PMWtr. In PMW, time since the final menstrual period did not differ ($p > 0.05$) between PMW groups (overall mean: 4.6 ± 0.7 years; see Table 1) and none were previous users of HRT. PMWtr participated in aerobic exercise >3 days/week for at least 5 years (mean ± SEM: 27 ± 4 years). Clinical characteristics of PreM hormonal contraceptive users ($n = 5$) did not differ ($p > 0.05$) from non-users ($n = 9$).

3.2 | Blood biomarkers

Basal SHBG, thyroxine, insulin, and glucose did not differ between groups ($p > 0.05$; Table 2). Estradiol and progesterone were higher ($p < 0.05$) and TC lower ($p < 0.05$) in PreM versus PMW groups. Testosterone was lower ($p < 0.05$) and LDLc higher ($p < 0.05$) in PMWun versus PreM. TG was lower ($p < 0.05$) in PMWtr versus PMWun and PreM. Triiodothyronine was lower ($p < 0.05$) and HDLc higher ($p < 0.05$) in PMWtr versus PreM.

Post-exercise nitrite concentrations (PreM, 68.2 ± 9.6 nM; PMWun, 90.6 ± 10.8 nM; PMWtr, 78.7 ± 12.7 nM) were unchanged ($p > 0.05$) from baseline in all 3 groups. Delta (post-exercise—pre-exercise) nitrite values (PreM, 13.0 ± 12.1 nM; PMWun, 10.9 ± 15.2 nM; PMWtr, 13.8 ± 11.8 nM) also did not differ ($p > 0.05$) between groups.

TABLE 1 Study group characteristics.

	PreM ($n = 14$)	PMWun ($n = 13$)	PMWtr ($n = 10$)
Age (years)	26 ± 1	56 ± 1†	57 ± 1†
Height (cm)	169 ± 2	164 ± 2	166 ± 2
Weight (kg)	63.7 ± 2.3	62.1 ± 2.1	58.7 ± 2.3
BMI (kg/m^2)	22.5 ± 0.7	23.2 ± 0.7	21.2 ± 0.4
$\dot{V}O_{2peak}$ (ml/kg/min)	39.8 ± 1.0	29.3 ± 0.5†	40.0 ± 1.1§
Calf circumference (cm)	35 ± 1	34 ± 1	33 ± 1
HR (bpm)	56 ± 2	59 ± 2	54 ± 2
Systolic BP (mmHg)	108 ± 2	117 ± 3	113 ± 2
Diastolic BP (mmHg)	72 ± 1	76 ± 1	73 ± 2
MAP (mmHg)	84 ± 2	89 ± 2	86 ± 2
Duration since FMP (years)*	–	4 ± 1	5 ± 1

Note: Data are expressed as mean ± SEM.

Abbreviations: BMI, body mass index; BP, blood pressure; FMP, final menstrual period; HR, heart rate; MAP, mean arterial pressure; $\dot{V}O_{2peak}$, peak oxygen uptake.

†vs PreM, $p < 0.05$.

§vs PMWun, $p < 0.05$.

*range 1–15 years.

3.3 | Calf blood flow

Pre-exercise, resting CBF and CVR were similar ($p > 0.05$) in PMW groups (Figure 1). In PMWun, resting CBF was lower ($p < 0.05$) and CVR higher ($p < 0.05$) compared with PreM, in whom values did not differ ($p > 0.05$) from PMWtr. Peak CBF was higher ($p < 0.05$) and CVR lower ($p < 0.05$) in PMWtr versus PMWun and PreM, in whom values did not differ ($p > 0.05$).

Post-exercise, between-group findings for resting and peak CBF and CVR were unchanged from pre-exercise. However, within groups, resting CBF increased ($p < 0.05$) and CVR decreased ($p < 0.05$) in all groups. Peak CBF increased ($p < 0.05$) and CVR decreased ($p < 0.05$) in PMW groups but remained unchanged ($p > 0.05$) in PreM.

3.4 | Hemodynamics

Pre-exercise, SBP, DBP, MAP, SV, CO, and TPR did not differ between the 3 groups ($p > 0.05$), but HR was higher ($p < 0.05$) in PMWun versus PMWtr (Table 3). Post-exercise, HR was higher ($p < 0.05$) in PMWun versus PMWtr, but other hemodynamic responses did not differ between groups ($p > 0.05$). Within groups, SBP, DBP, MAP, and SV were reduced ($p < 0.05$) in PMW but remained unchanged ($p > 0.05$) in PreM. Post-exercise HR and CO were increased ($p < 0.05$) in all groups. However, the relative increase in CO was lower ($p < 0.05$) in PMW (pooled groups) versus preM (10.5 ± 2.1% versus 18.8 ± 2.5%, respectively).

3.5 | Correlations

Pre-exercise, using pooled data (e.g., all groups), CBFpk was associated ($p < 0.05$) positively with HDLc (r = 0.549), and inversely with TG ($r = -0.416$) and insulin ($r = -0.456$). Conversely, CVRpk was associated ($p < 0.05$) inversely with HDLc ($r = -0.431$), and positively with TG ($r = 0.420$) and insulin ($r = 0.374$). Using separate groups for analysis, in PMWun only, CBFpk was associated ($p < 0.05$) inversely with glucose ($r = -0.717$) and positively with HDLc ($r = 0.633$; see Figure 2), with CVRpk associated positively with glucose ($p < 0.05$; $r = 0.740$). CVRpk also trended ($p = 0.05$) toward significant positive associations with LDLc ($r = 0.568$) and TG ($r = 0.553$). In contrast, in PMWtr, CBFpk was associated inversely ($p < 0.05$, $r = -0.725$) and CVRpk positively ($p < 0.05$, $r = 0.693$) with insulin only (see Figure 2). When using PreM data, CBFpk and CVRpk were not associated ($p > 0.05$) with circulating biomarkers.

Post-exercise, correlations were unchanged from pre-exercise. Using pooled data, CBFpk and CVRpk remained significantly ($p < 0.05$) associated with HDLc ($r = 0.664$ and $r = -0.601$, respectively), TG ($r = -0.489$; $r = 0.470$), and insulin ($r = -0.473$; $r = 0.388$). In PMWun only, CBFpk also remained associated ($p < 0.05$) negatively with glucose ($r = -0.721$) and positively with HDLc ($r = 0.679$), and CVRpk associated ($p < 0.05$) positively with glucose, LDLc and TG ($r = 0.740$, $r = 0.568$ and $r = 0.554$, respectively). In PWWtr, insulin remained associated ($p < 0.05$) inversely with CBFpk ($r = -0.683$) and positively with CVRpk ($r = 0.693$). In PreM, CBFpk and CVRpk persisted

TABLE 2 Baseline blood biomarkers of the study groups.

	PreM ($n = 14$)	PMWun ($n = 13$)	PMWtr ($n = 10$)
Estradiol (pmol/L)	184.6 ± 33.9	71.1 ± 0.7[†]	79.4 ± 5.7[†]
Progesterone (nmol/L)	1.6 ± 0.2	0.8 ± 0.1[†]	0.7 ± 0.1[†]
Testosterone (nmol/L)	1.1 ± 0.1	0.7 ± 0.1[†]	1.0 ± 0.1
SHBG (nmol/L)	68.9 ± 11.3	57.8 ± 5.6	59.8 ± 4.8
Triiodothyronine (pmol/L)	5.0 ± 0.1	4.6 ± 0.1	4.5 ± 0.1[†]
Thyroxine (pmol/L)	14.6 ± 0.5	13.8 ± 0.4	14.2 ± 0.5
TC (mmol/L)	4.6 ± 0.2	5.7 ± 0.2[†]	5.8 ± 0.3[†]
HDLc (mmol/L)	1.7 ± 0.1	2.1 ± 0.1	2.5 ± 0.2[†]
LDLc (mmol/L)	2.3 ± 0.1	2.9 ± 0.2[†]	2.6 ± 0.1
TG (mmol/L)	0.9 ± 0.1[§]	1.0 ± 0.1[§]	0.7 ± 0.1
Insulin (pmol/L)	31.0 ± 3.2	30.4 ± 2.8	23.2 ± 3.0
Glucose (mmol/L)	4.7 ± 0.1	4.8 ± 0.1	5.0 ± 0.1
Nitrite (nM)[a,b]	68.5 ± 15.9	79.6 ± 13.9	64.9 ± 7.3

Note: Data are expressed as mean ± SEM.

Abbreviations: HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; SHBG, sex hormone binding globulin; TC, total cholesterol; TG, triglycerides.

[a]data log-transformed for statistical analysis.

[b]PreM $n = 13$.

[†]vs PreM, $p < 0.05$.

[§]vs PMWtr, $p < 0.05$.

in not being associated ($p > 0.05$) with circulating metabolic or hormonal biomarkers.

Plasma nitrite concentrations were not significantly associated ($p > 0.05$) with CBF measures using pooled or sub-grouped data, or before or after exercise. Using delta (post-exercise minus pre-exercise) pooled and sub-grouped data, delta nitrite was also not associated ($p > 0.05$) with delta CBF measures. In PMW, time since menopause was not associated ($p > 0.05$) with absolute or delta CBF measures, nor circulating biomarkers.

4 | DISCUSSION

The novel findings of this study are that compared with healthy untrained age-matched PMW and younger PreM, habitually aerobically trained PMW demonstrate higher peak CBF and lower peak CVR, both before and 1 h after exercise. Further, we also report for the first time that despite similar circulating levels of glucose, insulin, HDLc, LDLc, and TC between our trained and untrained PMW, CBFpk and CVRpk were differentially associated with circulating metabolic factors. In trained PMW only, CBFpk was associated inversely, and CVRpk positively, with insulin. Conversely, in untrained PMW, CBFpk was associated inversely with circulating glucose and LDLc, and positively with HDLc, with CVRpk associated positively with TG, LDLc, and glucose. In contrast, in younger healthy untrained PreM, CBFpk and CVRpk were not associated with any circulating metabolic biomarkers. Collectively, these findings suggest a habitual aerobic exercise in PMW may augment peripheral blood flow and lower regional vascular resistance through favorable modulation of vasculo-metabolic interactions, including augmented vascular sensitivity to insulin and/or attenuated adverse vasomodulation by circulating proatherogenic lipids.

4.1 | Pre-exercise vascular measures

For the first time, we report that compared with untrained PMW and PreM, habitually aerobically trained PMW demonstrate higher CBFpk and lower CVRpk in response to reactive hyperemia. This finding is consistent with one study demonstrating increased peak CBF in older (~65 years) men and women after ~31 weeks of walking/jogging training.[12] Other lower-limb studies in PMW also report augmented femoral artery blood flow after short-term (12 weeks)[15] and habitual[23] exercise training in PMW in the early[15] and late[23] stages of menopause. Collectively, these[12,15,23] and the current findings suggest that short-term to chronic aerobic exercise training elicits beneficial vascular adaptations in the micro- and macro-vasculature of the lower limb in PMW. Augmented synthesis of, and smooth muscle sensitivity to, the vasodilator prostacyclin,[15,23] angiogenesis,[23] and elevated levels of endogenous antioxidative enzymes[23] are implicated as potential mechanisms.

In the current study, we extend on the possible mechanisms of peripheral blood flow in PMW to include vasculo-metabolic interactions. Namely, despite 'normal', and similar, circulating levels of metabolic markers in our PMW groups, including glucose, insulin, LDLc, and HDLc, CBFpk was associated negatively with insulin in PMWtr only. This finding suggests greater vascular sensitivity to insulin in trained compared with PMWun. This notion is consistent with studies reporting augmented endothelial insulin sensitivity in endurance-trained individuals in association with greater insulin-mediated NO production via the PI3K/Akt pathway.[24] In contrast, in our PMWun, CBFpk was not associated with insulin, rather it was associated inversely with circulating glucose, LDLc, and positively with HDLc. Whether vascular sensitivity to insulin-mediated vasodilation is decreased in PMWun, and/or vascular sensitivity to circulating lipids or glucose is increased, the observed vasculo-metabolic findings in

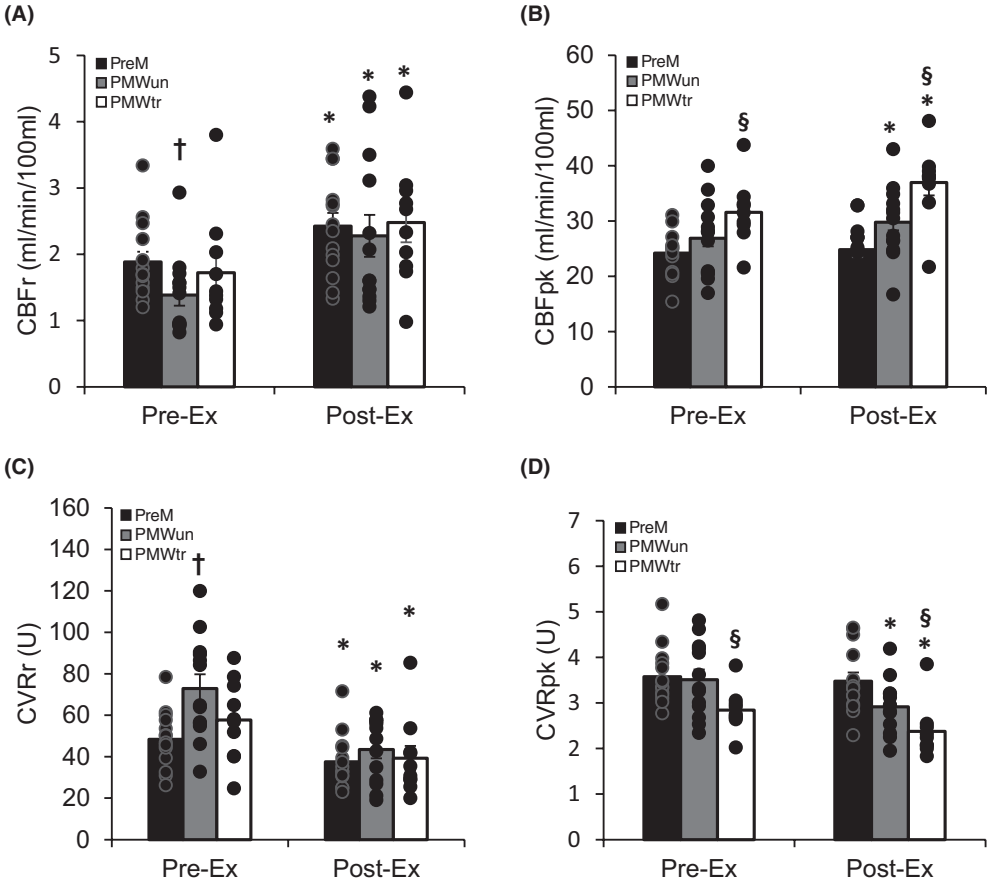

FIGURE 1 Resting and peak calf hemodynamics pre- and post-exercise. A and B: Resting and peak ischemic CBFr and CBFpk, respectively, before and after exercise. C and D: Resting and peak ischemic CVRr and CVRpk, respectively, before and after exercise. Data are expressed as mean ± SEM. †vs PreM, $p < 0.05$, within condition. §vs all other groups, p-exercise, $p < 0.05$, within condition. * vs pre-exercise, $p < 0.05$, within group. CBFpk, peak calf blood flow; CBFr, resting calf blood flow; CVCpk, peak calf vascular conductance; CVCr, resting calf vascular conductance; CVRpk, peak calf vascular resistance; CVRr, resting calf vascular resistance.

TABLE 3 Hemodynamic measures before and after exercise.

	PreM ($n = 14$)		PMWun ($n = 13$)		PMWtr ($n = 10$)	
	PreEx	**PostEx**	**PreEx**	**PostEx**	**PreEx**	**PostEx**
HR (bpm)	56 ± 2	65 ± 2*	59 ± 2	69 ± 1*	54 ± 2§	61 ± 2§,*
Systolic BP (mmHg)	108 ± 2	106 ± 2	117 ± 3	108 ± 3*	113 ± 2	109 ± 2*
Diastolic BP (mmHg)	72 ± 1	72 ± 2	76 ± 1	71 ± 2*	73 ± 2	71 ± 2*
MAP (mmHg)	84 ± 2	83 ± 2	89 ± 2	83 ± 2*	86 ± 2	84 ± 2*
SV (ml)[a]	70 ± 3	72 ± 3	70 ± 2	67 ± 2*	72 ± 4	70 ± 4*
CO (l/min)[a]	3.9 ± 0.2	4.6 ± 0.3*	4.0 ± 0.2	4.4 ± 0.2*	3.9 ± 0.1	4.3 ± 0.3*
TPR (dyn/s per cm^5)[a]	1791 ± 89	1493 ± 80*	1814 ± 77	1518 ± 66*	1843 ± 154	1625 ± 102*

Note: Data are mean ± SEM.

Abbreviations: BP, blood pressure; CO, cardiac output; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance.

[a]PMWun, $n = 12$; PMWtr, $n = 8$.

*vs pre-exercise, within group, $p < 0.05$.

§vs PMWun, within condition, $p < 0.05$.

PMWun are consistent in part with the known vasoconstricting effects of circulating proatherogenic lipids and glucose.[25–27] In contrast, no vasculo-metabolic interactions were observed in PreM. Taken together, our findings suggest that estrogen deficiency due to menopause may independently mediate a shift toward vasomodulation

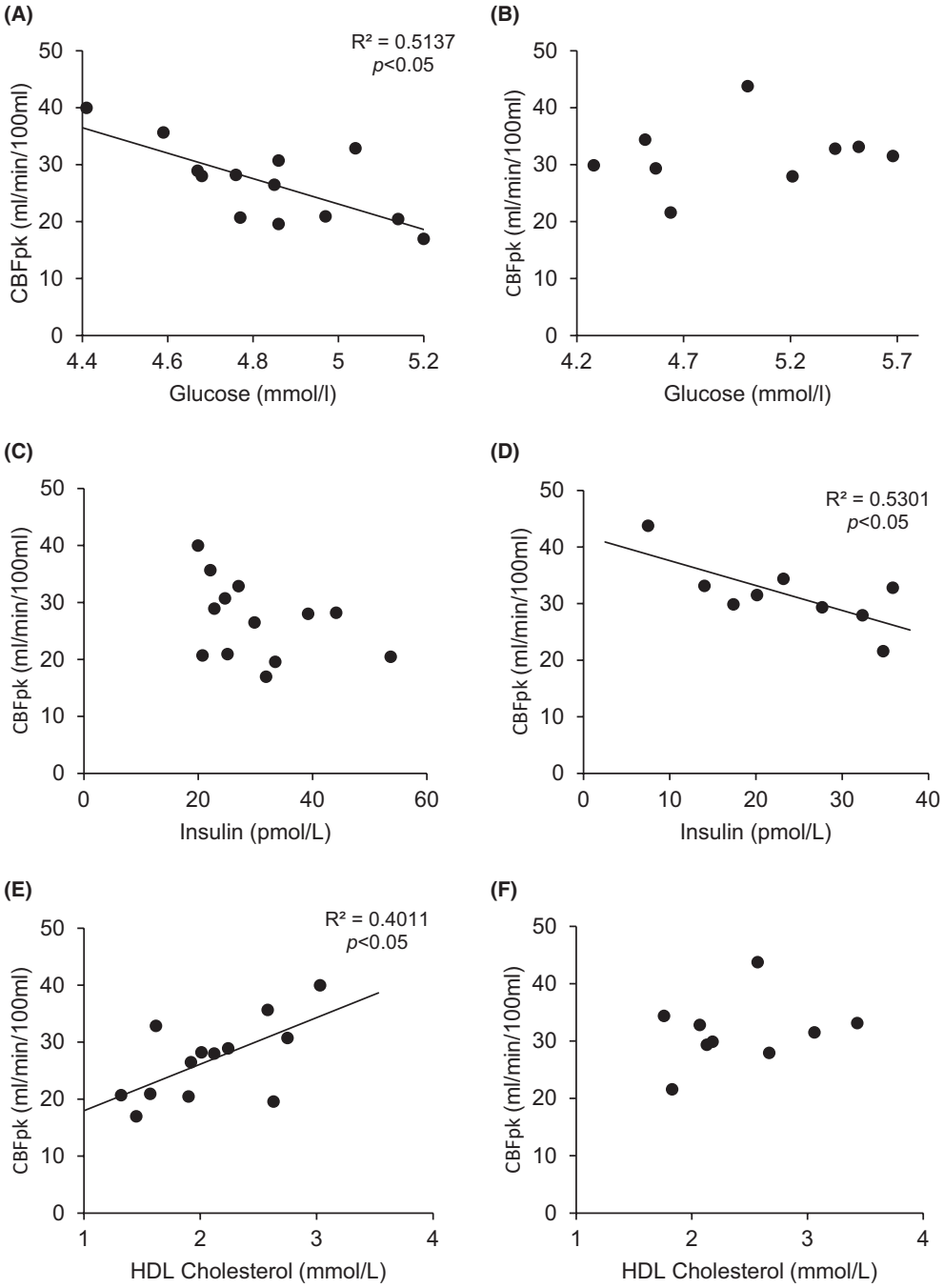

FIGURE 2 Correlations between peak calf blood flow (CBFpk) and circulating cardiometabolic markers in untrained and trained PMW. A and B: CBFpk and glucose in PMWun and PMWtr, respectively. C and D: CBFpk and insulin in PMWun and PMWtr, respectively. E and F: CBFpk and HDL cholesterol in PMWun and PMWtr, respectively. HDL, high-density cholesterol.

by circulating lipids and glucose, with exercise training potentially offsetting these effects during the postmenopausal period, possibly in association with increased vascular sensitivity to insulin. Further investigations with larger studies are needed to confirm these hypotheses.

It is pertinent to acknowledge that the current study findings are in contrast to those reporting no impact of exercise training on endothelial function in the popliteal[28] and brachial[3,13,14] arteries, and the forearm microvasculature[14] in PMW. Causes of discrepancy between vascular findings are unclear. However, methodological and physiological differences likely play a role, including the use of reactive hyperemia versus flow-mediated dilation, assessment of different vascular beds, and use of the trained versus untrained limb. In the current study, all PMWtr were predominantly runners. Thus, we assessed vascular function in the trained lower limb. Repeated bouts of dynamic exercise have

been shown to induce numerous vascular adaptations in the skeletal muscle vascular bed, including increased tissue capillarization and small arteriole density,[29] outward remodeling of arterioles and feed vessels,[30] and improved functioning of the endothelium and/or smooth muscle cells of blood vessels.[29] Such adaptations predominantly occur in response to local, rather than systemic, stimuli.[31] This may be of relevance to studies that have reported no effects of exercise training on macro- and microvascular endothelial function in the untrained upper limb in PMW predominantly participating in lower limb-based activities (e.g., running or cycling).[3,13,14] In contrast, however, middle-aged male runners demonstrate improvements in brachial artery endothelial function in the untrained upper limb.[9,11,13] Exact causes of sex differences in vascular adaptations to exercise training remain to be elucidated.

4.2 | Vascular responsiveness to acute dynamic exercise

Sustained post-exercise vasodilation in the skeletal muscle vascular beds is well documented after whole body and small muscle mass exercise.[32] In keeping with this, we report that 1 h after an acute bout of aerobic exercise, all groups demonstrated increased resting CBF and decreased CVR. However, peak CBF and CVR responses were increased and decreased, respectively, within PMW groups only. Between groups, vascular responses did not differ between untrained PMW and PreM, yet CBF remained higher and CVR lower in trained PMW. Although no studies to date have reported post-exercise CBF in untrained and habitually aerobically trained PMW, this finding is consistent with a previous study in sedentary healthy pre- and post-menopausal women reporting similar decreases in CVR after 45 minutes of dynamic exercise.[33] Increased peripheral blood flow and reduced vascular resistance after an acute bout of exercise are known to be mediated through neural and endothelial mechanisms, including reduced outflow of sympathetic vasoconstrictor nerve activity to the vasculature of skeletal muscle[34] and increased synthesis of vasoactive molecules, including histamine,[35] prostaglandins[36] and NO.[37,38] In keeping with this, we postulated that exercise would increase CBF in all groups in association with increased circulating NO levels. However, we failed to detect increases in plasma nitrite concentrations (a biomarker of NO availability) one hour after exercise in any of our groups. The reasons for this observation are unclear. However, timing of assessment,[32] assessment of NO versus one of its more stable metabolites,[39] and differences in tissue versus circulating levels[39] may play a role.

Our study compared vascular function in trained and untrained women using a prescribed exercise stimulus that was relative to the individuals' cardiorespiratory fitness (i.e., the same relative exercise intensity). While this individualized approach is commonly used for the assessment, monitoring, and enhancement of cardiorespiratory fitness,[40] it is appropriate to acknowledge that exercising at a relative intensity may result in differing absolute metabolic (e.g., a metabolic equivalent [MET]) and/or cardiovascular (e.g., HR) strain, particularly when comparing trained versus untrained individuals. A MET is a common method used to objectively quantify physical activity intensity, using a ratio of the metabolic cost produced by different types of exercise and intensity compared to the metabolic cost of sitting quietly (e.g., 3.5 mL/kg/min). Moderate exercise intensity such as walking is categorized 3–6 METs and vigorous exercise such as running is >6 METs.[40] In contrast to relative exercise intensity, absolute intensities are independent of the physiological capacity of the individual.[41] Thus, in individuals with high aerobic capacity, exercise intensity may be overestimated when using MET cut-points.[41] In keeping with this, we observed higher METs (~7) when exercising (walking) at the same relative intensity in the trained PMW and untrained PreM groups compared to the untrained PMW (~5METs), suggesting higher absolute exercise intensity in the trained PMW and untrained PreM groups compared to the untrained PMW. Lack of agreement between relative and absolute exercise intensities, particularly with walking, as employed in the current study, has been previously reported.[40] The effects of relative versus absolute exercise intensity on vascular function in PMW remain to be determined and warrant further research.

In addition to lower limb vascular responses to an acute bout of dynamic exercise, we also assessed systemic hemodynamic responses. In PMW, post-exercise BP and TPR were significantly reduced, and CO increased from pre-exercise values. In PreM, BP was unchanged, yet TPR decreased and CO increased. These findings are consistent with previous studies.[33] While PreM and PMW groups demonstrated a similar decline in TPR, the greater offsetting increase in CO observed in PreM may have contributed to their unchanged BP. Similar to changes in local vascular resistance, the mechanisms underpinning systemic reductions in vascular resistance may involve neural and vascular components. Consistent with previous findings in healthy young men and women,[42] we report that prolonged post-exercise hypotension in PMW does not appear to be dependent on increased circulating NO

levels, suggesting other factors may play a role. Studies to examine these mechanisms in PMW await investigation.

4.3 | Limitations

Inclusion of only healthy, recently menopausal women (~5 years on average), means we cannot extrapolate our findings to other groups of postmenopausal women, including women in the earlier or later stages of menopause, or women presenting with chronic disease. Strain-gauge plethysmography is a validated and well-recognized method to assess vascular function. However, other methods, such as ultrasound imaging techniques, would have provided additional information regarding vascular structure and function. Our chosen protocol may have benefitted from additional assessments post-exercise to allow us to observe the time course of the effects of exercise on vascular function. We investigated the effects of relative, but not absolute, exercise intensity on vascular function. This limited our ability to compare possible differences in vascular function in response to the same absolute exercise intensity and how this differed from the same relative exercise intensity in our population groups. The estimation of the formation of endogenous NO includes an assessment of its more stable oxidation products, nitrate, and nitrite. We assessed nitrite but not nitrate, and as such, this may have limited our NO-vascular findings. Calf circumference did not differ between our study groups. However, we did not assess the composition of the calf, which may have differed between groups and possibly contributed to differences in CBF. Our investigations were cross-sectional in nature, therefore, causal links could not be established. Finally, the use of small sample sizes may have prevented us from detecting true differences when they were present. Taken together, our findings should be interpreted accordingly.

5 | CONCLUSIONS

Compared with untrained PMW, habitually aerobically trained PMW demonstrate higher peak CBF and lower CVR in association with circulating insulin levels. In contrast, despite similar concentrations of LDLc, TC, and glucose between PMW groups, these biomarkers were associated inversely with CBF, and positively with CVR, in untrained PMW only. Conversely, no vasculo-metabolic interactions were observed Iin PreM. Collectively, these findings suggest that impaired vascular function in estrogen-deficient PMW may, in part, be associated with a shift toward vasomodulation by circulating lipids and glucose and that aerobic exercise training may potentially mitigate this shift. While the clinical relevance of these findings is unknown, reduced peripheral blood flow is mechanistically implicated in the evolution of metabolic syndrome,[43] the prevalence of which is known to increase after menopause.[1] Aerobic exercise training may therefore help lower the risk of developing metabolic syndrome and cardiovascular disease in PMW, in part, by augmenting peripheral blood flow in association with favorable modulation of metabolic-vascular interactions. Larger studies to confirm these postulates are warranted.

6 | PERSPECTIVES

The finding of differing vasculo-metabolic interactions in aerobically trained versus untrained PMW is new and potentially provides novel clinical perspectives into the cardiovascular effects of menopause, and the modulatory effects of exercise. Circulating proatherogenic lipids and glucose, even within the normal healthy range, may unfavorably modulate vascular function in untrained but otherwise healthy PMW, with habitual aerobic exercise training potentially "off setting" or mitigating these vasculo-metabolic interactions. Given the key role of vascular dysfunction in the pathogenesis of metabolic syndrome and atherosclerosis, and the known link between estrogen and vascular and metabolic health in PMW,[1,3,4,44] these findings contribute to the understanding of the effects of menopause on peripheral blood flow in PMW, and the potential modulatory effect of exercise training. Larger studies to confirm these findings are needed.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT

All participants provided written consent prior to study participation.

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