Brislane, Á, Low, DA, Carter, SE, Holder, SM, Jones, H and Hopkins, ND

Cerebral and peripheral vascular differences between pre- and post-menopausal women

Article

FULL TITLE: CEREBRAL AND PERIPHERAL VASCULAR DIFFERENCES BETWEEN PRE- AND POST-MENOPAUSAL WOMEN

SHORT TITLE: Menopause and the vascular system

Áine Brislane, PhD.[1,2]
David A. Low, PhD[1]
Sophie E. Carter, PhD[1,2]
Sophie M. Holder, MPhil[1]
Helen Jones, PhD[1]
Nicola D. Hopkins, PhD[1]

[1] Research Institute of Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom.
[2] School of Sport, York St. John University, York, United Kingdom.

Sources of financial support: None
Conflict of Interest: None
Disclaimers: None

Figures: 3 **Tables:** 3

Corresponding Author: Dr. Áine Brislane, School of Sport, York St. John University, Lord Mayor's Walk, York, YO31 7EX, United Kingdom. Tel: +441904 876795, E-mail: a.brislane@yorksj.ac.uk

Abstract

Objective: Menopause is associated with lower peripheral vascular function however cerebrovascular responses to this time-period are unclear. We aimed to describe peripheral vascular and cerebrovascular differences between pre- and post-menopausal women.

Methods: Fifty pre- and post-menopausal women (N=100) underwent assessments of cerebral blood flow, cerebrovascular reactivity and autoregulation, carotid artery reactivity, brachial and femoral artery flow-mediated dilation and carotid, brachial and femoral artery intima-media thickness. Comparisons were made between pre- and post-menopausal women followed by a secondary-analysis (N=20) between late-pre-menopausal women and those within five years of menopause using a general linear model.

Results: Cerebral blood flow (-11 [-17, -4 cm/s]; p=0.03) and carotid reactivity (-2.3 [-4.3, -0.3%] p=0.03) were lower post-menopause compared to pre-menopause while cerebrovascular reactivity and autoregulation did not differ (p>0.05). Post-menopausal women had a larger carotid (0.16 [0.13, 0.20 mm] p<0.001), brachial (0.07 [0.03, 0.11mm] p=0.004) and femoral artery intima-media-thickness (0.09 [0.05, 0.14 mm] p=0.04), alongside lower brachial (-2.3 [-3.9, -0.7%] p=0.004) and femoral artery flow-mediated dilation (-3.0 [-4.3, -1.8 %] p<0.001). In the secondary-analysis, early-post-menopausal women had a lower femoral artery flow-mediated dilation (-1.9 [-3.9, -0.0 %] p=0.05) and larger carotid intima-media-thickness (0.07 [0.00, 0.14 mm] p=0.03) compared to late-pre-menopausal women.

Conclusions: Cerebral blood flow, carotid artery reactivity, peripheral vascular function and structure are negatively affected by age. Preliminary data indicates that femoral artery function and carotid artery structure may be potentially impaired in early-post-menopause compared with late-pre-menopause. These findings suggest that conduit arteries susceptible to atherosclerosis may be important targets for lifestyle intervention in early menopause.

Key words: Menopause, cerebrovascular, flow-mediated dilation, endothelial function.

Introduction

Age is an independent determinant of cardiovascular disease (CVD) [1] and is accompanied by increased traditional CVD risk factors, including body mass index (BMI), blood pressure (BP), and cholesterol levels [2]. For women, increased CVD risk coincides with the onset of menopause, characterised by a reduction in the sex hormone oestrogen [3]. As a result, the menopause is associated with a greater decline in peripheral vascular function compared to pre- and peri-menopause via diminished nitric oxide bio-availability at the endothelium [4]. However, the functional and structural adaptations that arise at various other vascular beds during and following the menopause remain unclear.

Ageing is associated with the incidence of cerebrovascular diseases such as vascular dementia [5] with greater prevalence in women compared to men [6]. It has been inferred that the menopause may contribute to cerebrovascular disease development [7,8] however, limited evidence regarding the additive effect of menopause and ageing on female cerebrovascular function makes this difficult to determine. Cerebral blood flow, as measured via middle cerebral artery velocity (MCAv), declines in ageing men [9] and is associated with CVD and neurodegenerative disease [10]. This may be different for women given that the cerebral blood vessels contain oestrogen receptors that participate in the release of nitric oxide facilitating vasodilation [11]. MCAv has shown to be comparable between pre-menopausal (PRE-M) and post-menopausal (POST-M) women, although some women included in this dataset were taking hormone therapy [12]. Furthermore, MCAv is reportedly unchanged in women following surgical menopause [13] implying that ageing and not the immediate oestrogen reduction to be responsible for reduced MCAv. Elsewhere however, characteristics of MCAv waveforms have been shown to differ between pre- and post-menopausal with potential to provide pathophysiological insight into cerebrovascular disease that accompanies age [14]. The evidence regarding cerebrovascular reactivity (CVR); a marker of cerebrovascular function, is conflicting having been reported as lower [12,15] and unchanged [16] in POST-M compared to PRE-M women. Similarly, cerebral autoregulation is reportedly impaired in POST-M compared to

PRE-M assessed using the breath hold index [12]. Contrastingly, cerebral autoregulation appears unaffected by menopause where elderly women have been compared to age matched men, implying no implication of oestrogen reduction on cerebral autoregulation [17]. However, no study has measured various parameters of cerebrovascular function in a large cohort of POST-M women to elucidate cerebrovascular health during the early and latter years of menopause. Contrasting findings between studies, different measurement techniques [12,16], small sample sizes [16] and liberal inclusion criteria such as elderly people with illnesses[15] and cohorts including women taking hormone therapy [16] prevent any meaningful consensus being reached on the effect of menopause on cerebrovascular function and thus, provide rationale for the current study.

Physical activity (PA) and exercise are important modifiable lifestyle factor that improves cardiorespiratory fitness, and enhances peripheral vascular function [18,19], MCAv [20] and CVR in post-menopausal women [21]. PA levels and fitness may therefore be critical confounders in any study which aims to assess vascular outcomes in POST-M women. Yet, no study has attempted to objectively quantify PA and CRF in conjunction with cerebral and peripheral vascular parameters between pre and post-menopausal women. Therefore, the primary aim of this study was to describe the peripheral and cerebrovascular changes that arise from pre- to post-menopause with simultaneous assessment of PA and CRF. We hypothesise that PRE-M women will have higher peripheral and cerebrovascular function compared with POST-M women. We also hypothesise that fitness and PA levels will be higher in PRE- compared with POST-M women.

Materials and Methods

Participants

Females (n=100) aged between 18-70 years were recruited from the local community through social media advertisements and word of mouth over an 18-month period between April 2016 and October 2017. Participants were non-smokers, had no history of diabetes, cardiovascular

disease (CVD) and were not on any form of medication. PRE-M women were defined as eumenorrheic having a consistent menstrual cycle for at least 3-months and were not on any form of hormone-based contraception. POST-M women were recruited based on having no menstrual cycle for at least 12 consecutive months and were not previously or currently taking any form of hormone therapy [4]. Each participant provided written informed consent before taking part in the experimental procedure. The research study was ethically approved by the Liverpool John Moores School of Sport and Exercise Science Research Committee (Reference: 16/SPS/022) and adhered to the Declaration of Helsinki.

Experimental Procedure

Participants visited the laboratory on two occasions following abstinence from exercise for 24 hours and alcohol for 12 hours, as well as any food/caffeine/stimulants six hours prior to the experiment. Participants completed a battery of CV and cerebrovascular assessments in the following sequence: intima-media thickness (IMT), flow-mediated dilation (FMD), pulse-wave velocity (PWV), carotid artery reactivity (CAR), CVR, and cerebral autoregulation, followed by seven days of PA and sedentary behaviour monitoring. A maximal CRF test on a cycle ergometer was scheduled within seven days of the vascular measurements based on participant's preference. All visits were completed at the same time of day (between 8-11am) in a temperature-controlled environment (20-22°C). Eumenorrheic women completed the laboratory visit within the first seven days of their menstrual cycle [22].

Anthropometrics, physical activity and sedentary behaviour

Anthropometry and Body Composition. Stature and weight were recorded to the nearest 0.1unit using a stadiometer and digital scales respectively. BMI was calculated as weight in kilograms divided by stature in metres squared (kg/m^2). Body fat percentage was estimated using bioelectrical impedance analysis (Tanita BC-420MA, Tanita Corp., Tokyo, Japan).

Cardiorespiratory Fitness Test. A ramp-based cycling protocol was used to determine maximum oxygen uptake ($\dot{V}O_{2max}$). Participants cycled until volitional exhaustion or, until

participants were no longer able to maintain a pedal speed of at least 50 rpm. Breath-by-breath oxygen uptake ($\dot{V}O_2$) and carbon dioxide (CO_2) were measured via an online gas analysis system (Jaeger Oxycon Pro, Viasys Health Care, Warwick, UK). Heart rate (HR) was monitored continuously using short-range telemetry (Polar, Kempele, Finland). Participants completed a 5-minute warm up at a self-selected resistance. The test began with 50 W of resistance and increased by 30 W every two minutes. During the final 20 seconds of each stage, participants were asked to rate their exertion, using the Borg rating of perceived exertion (RPE) scale [23], and HR was recorded. Criteria for participants reaching their maximal capacity was achieving a respiratory exchange ratio of >1.15, a HR >199 bpm and/or an RPE of 20 on the Borg RPE Scale [24]. The $\dot{V}O_2$ data was exported in 10 second averages for the duration of the test to an Excel file and plotted on a graph with time on the x-axis and $\dot{V}O_2$·ml·kg·min on the y-axis. The highest value over 30 seconds was extracted as the $\dot{V}O_{2max}$ value.

Physical Activity (PA). PA was monitored using a tri-axial accelerometer (Actigraph wGT3x-BT, Pensacola, Florida) worn on the right hip for consecutive days. Participants removed the device for sleeping, contact sports and water-based activities and recorded the times the device was worn on a diary sheet provided. Non-wear time was defined as 90 consecutive minutes of zero counts·min^{-1} [25]. Inclusion criteria for analysis were ≥10 hours of wear time per day, for a minimum of four days, including one weekend day [26]. Actilife software, version 6.2 (ActiGraph, Pensacola, Florida) was used to download the data from the accelerometer to a computer. Raw acceleration data was converted to 60s-epoch activity count data (counts·min^{-1}). PA intensity was determined using the following cut points [27]: light (≤2689 counts·min^{-1}), moderate (≤6166 counts·min^{-1}), and vigorous (>6167 counts·min^{-1}). Activity data were exported and handled in Excel (Microsoft) and total time (minutes) spent in light, moderate and vigorous intensity PA was calculated. To account for any differences in accelerometer wear time, PA data were also expressed as a percentage of wear time.

Sedentary Behaviour. Sedentary time was objectively measured using an activPAL activity monitor (activPAL micro, PAL Technologies Ltd., Glasgow, UK) worn continuously for seven days on the middle anterior of the right thigh. Monitors were enclosed in a rubber sleeve and attached by the researcher to the skin using a waterproof transparent seal (Tegaderm Roll, 3M). The monitor quantified the time spent sitting/lying per day. A sedentary bout was defined as no activity registered for at least 60 seconds [28]. The raw data was downloaded from the monitor using the activPAL proprietary software (version 7.2.32) from which data were exported to Excel. Average daily sedentary behaviour was calculated in seconds during self-reported waking hours and converted to minutes.

Cerebrovascular Measurements

Middle Cerebral Artery Blood Flow Velocity (MCAv). MCAv was measured at rest using a 2 MHz pulsed transcranial Doppler (TCD) ultrasound system (DWL, Compumedics, Germany). The participant was fitted with a headband which supported an ultrasound probe on each side of their head. Ultrasound gel was applied to the temporal window (just above the zygomatic arch) and to the probes allowing an optimal signal to be obtained. To insonate the correct vessel, specific criteria were followed with the mean and peak MCAv values above 50 $cm \cdot sec^{-1}$ and 80 $cm \cdot sec^{-1}$ respectively and depth set between 40-60 mm [29]. Care was taken to stabilise the probes ensuring a consistent angle of insonation, in line with best practice guidelines [30,31]. Simultaneously, arterial BP was monitored non-invasively using a photoplethysmographic cuff (Finapres Medical Systems, Enschede, Netherlands) which was carefully fitted on the middle or index finger of the participant's right hand as per manufacturer's recommendations. Real time MCAv and BP were recorded online and displayed in LabChart Pro version 7 (ADInstruments, Colorado Springs, CO). The weighted mean MCAv was calculated from the peak envelope of the velocity trace (1/3 systolic + 2/3 diastolic), which accounts for the relative time spent in each phase of the cardiac cycle [32]. Data were expressed as cerebrovascular conductance (CVC), which was calculated as MCAv divided by mean arterial pressure (1/3 systolic BP + 2/3 diastolic BP). Unilateral, or when

obtainable, bilateral MCAv was recorded during all cerebral tests. Mean MCAv was calculated when bilateral MCAv was recorded.

Cerebrovascular Reactivity (CVR). Cerebrovascular reactivity to perturbations in partial pressure of oxygen ($PaCO_2$) was measured using a hypercapnic rebreathing protocol [32]. Participants were instrumented as above with the addition of a rebreathing apparatus consisting of a mouthpiece, nose clip, a bacteriological filter and a three-way valve to allow switching of airflow between room air and a pre-filled Douglas bag containing a hypercapnic gas mixture of 5% CO_2 and 21% O_2, balanced with nitrogen. Breath-by-breath CO_2 was sampled using a calibrated gas analyzer (Ml206, ADInstruments, Oxford, UK) and the pressure of end-tidal carbon dioxide ($PETCO_2$) was recorded online (LabChart) and corrected for the daily barometric pressure. A one-minute baseline recording was followed by a period of voluntary hyperventilation (1 breath per second) coached by the researcher until a reduction in $PETCO_2$ to <20 mmHg. Once achieved, the valve on the Douglas bag was switched so participants inhaled the 5% CO_2 mixture. Simultaneously, participants were instructed to return their respiratory rate to normal whilst breathing the 5% CO_2 mixture for 3-minutes. Data was exported from LabChart.

Baseline $PETCO_2$ and MCAv were calculated as the mean of the 1-min prior to hyperventilation, while MCAv and $PETCO_2$ data during 5% CO_2 breathing was collected as 10-second averages for the entire 3-minute period. Absolute MCAv and relative changes were plotted against $PETCO_2$ for each 10-sec of 5% CO_2 of breathing and reported as absolute and relative CVR sensitivity (cm/s per mmHg). CVR was subsequently quantified by linear regression (R^2 value). Relative MCAv was calculated as the difference between baseline and 5% CO_2 MCAv divided by baseline MCAv (([5% CO_2 MCAv-baseline MCAv]/ baseline MCAv) x 100%) [32,33]. Simultaneously, during the baseline and CO_2 breathing measurements, arterial diameter and blood velocity of the left common carotid artery (CCA) were acquired at least two centimeters below the point of bifurcation using high resolution ultrasound. Images were acquired in accordance with methodological guidelines [34]. Data were used to determine the

response of the CCA to elevations in $PETCO_2$ by averaging 30-sec of baseline diameter and comparing that to the diameter during the last 30-sec of 5% CO_2 breathing.

Cerebrovascular Autoregulation. Changes in BP and MCAv were assessed using a squat to stand procedure in order to induce transient changes in BP. Participants performed squat-stand maneuvers at 0.10 Hz (5 second squat - 5 second stand) whilst breathing normal atmospheric air for a duration of 6 minutes with $PETCO_2$ monitored throughout. MCAv, $PETCO_2$, and beat-to-beat MAP were extracted from LabChart across the 6-minute period. The relationship between changes in MCAv and arterial BP was assessed via transfer function analysis (TFA) in accordance with standardized guidelines [35]. TFA was performed using MATLAB (MathWorks-Inc., Natick, MA) in order to calculate associated power (gain) timing (phase) and timing normalized to blood pressure (normalized gain) over three different frequencies; very low (0.02 – 0.07 Hz), low (0.07 – 0.20 Hz) and high (0.20 – 0.50 Hz) [35]. Gain rises with increasing BP frequencies and when increased, is indicative of diminished dynamic cerebral autoregulation efficiency [36]. The second cerebral autoregulation parameter, phase, describes the synchronicity of oscillations of BP and MCAv, and a greater phase indicates more efficient cerebral autoregulation whereby MCAv and BP waveforms are in sync with one another [36]. TFA also produces an estimated reliability of the relationship between the two signals (coherence) [37]. Data sets with a coherence value of <0.4 were excluded from data analysis. High frequency and very low frequency range data were excluded from analysis based on the frequency of the squat-stands used.

Carotid Artery Reactivity

In a supine position, left CCA diameter response was measured via ultrasound with participant's neck slightly extended to facilitate adequate imaging. A one-minute baseline measurement was recorded, then participants were instructed to immerse their left hand up to the wrist into a bucket of icy water (1-5ºC) for three minutes. For the duration of this test, participants were encouraged to breathe normally (avoid breath holding/hyperventilation) and

to keep as still as possible, without speaking [38]. Post immersion CCA data was assessed at 10-second intervals using the custom designed edge-detection and wall tracking software (Dicom Encoder) from which peak diameter change and area under the curve for the diameter change during cold pressor test (CAR_{AUC}) was calculated. The peak diameter change refers to dilation or constriction, and the direction of this change was determined by a positive or negative CAR_{AUC} (i.e. dilation or constriction respectively). The technique shows a good correlation between the CCA diameter response (an extracranial blood vessel) and coronary artery flow response in asymptomatic healthy adults, indicating the carotid artery is a valuable site in assessing vascular health [38]. Taken together, this test can provide information on coronary artery vasodilator function and could be a valuable link to evaluating systemic vascular health with the menopause and aging in women.

Cardiovascular measurements

Carotid, Femoral and Brachial Intima-Media Thickness. Following 20 minutes of rest in a supine position, the left CCA was imaged using high-resolution B-mode ultrasound (Terason u-smart 3300, Teratech, Burlington, MA) with a 10-12 MHz linear array probe 5 mm proximal to the artery bulb [39]. Participants lay with neck slightly extended facing the contralateral side to allow for optimal longitudinal imaging of the far-wall intima media interface from three angles (approximately 45°, 90° and 135°). Images were optimised to ensure clear contrast between the artery walls and lumen with a distinct IMT visualised on the far wall defined as the distance between two echogenic lines represented by the lumen-intima interface and media adventitia interface of the artery wall. The IMT was also acquired at the left femoral artery 3 to 5 cm distal to the bifurcation of the femoral artery and left brachial artery 5 to 10 cm above the elbow and using the same criteria as for CCA. Each image was recorded by the same sonographer for 30-40 seconds. Due to data quality issues, brachial IMT was only obtainable for N=64 women. Recordings were analysed offline using the edge detection software Carotid Studio v4.3.1 (Cardiovascular Suite, Quipu srl, Pisa, Italy) with a frame rate of 25 frames per second. Following calibration, an optimal region of interest that included both vessel walls with a

minimum length of 1 cm was selected by the researcher. Based on the quality of the scan, a 5-15 second time frame was chosen for analysis. The automated software produced an edge detection output of the near and far-wall media-adventitia interface during each cardiac cycle using a pixel-density algorithm. Continuous calculations by the software produced an average IMT and artery diameter recorded within the operator selected time duration. This was repeated for all three angles and an average of the three angles was calculated. This method has been shown to be valid and reproducible [40,41]. The extracted data were also used to calculate wall-to-lumen ratio (IMT/Lumen) at each arterial site which corrects for differences in baseline diameter.

Arterial Stiffness. Carotid-femoral PWV was assessed using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia) in the supine position. Firstly, three brachial BP measurements were taken in succession (Dinamap V100, GE Medical Systems, Germany), with an average systolic (SBP) and diastolic (DBP) calculated and entered into the software. A single applanation tonometer probe was used to capture a proximal (carotid artery) and distal (femoral artery) pulse, recorded over 10 cardiac cycles. The QRS complex was measured simultaneously using electrocardiography (ECG). The time between the R wave of the ECG trace and the foot of the proximal waveform is subtracted from the time between the R wave and the foot of the distal waveform to obtain the pulse transit time. To determine the distance used for PWV, the distance from the proximal measurement site to the suprasternal notch was subtracted from the distance between the distal measurement site and the suprasternal notch using an anthropometric measuring tape. PWV was automatically calculated by dividing the distance between the two arterial recording sites by transit time to provide an index of arterial stiffness. PWV measurements were made in triplicate and an average was calculated and used in data analysis.

Brachial and Femoral Flow Mediated Dilation. Left brachial and left femoral artery diameters were assessed simultaneously via high resolution 2D duplex ultrasound as per the IMT assessments. B-mode images were obtained and optimised, and the probe was held in the

same position for the duration of the test. After 1-minute of baseline measurement, occlusion cuffs, connected to a rapid inflator (Hokanson, Bellevue, WA), placed around the left thigh, proximal to the patella, and the left forearm, distal to the humeral epicondyle, were inflated to a supra-systolic pressure of 220 mmHg for 5-minutes. Arterial images were recorded for a further 3-minutes post cuff deflation in accordance with best practice guidelines [42,43]. Brachial FMD (bFMD) and femoral FMD (fFMD) data were analysed by custom designed edge-detection and wall tracking software (Dicom Encoder), of which the reproducibility and validity have been demonstrated elsewhere [44]. An optimal region of interest was selected by the sonographer, on the basis of the quality of the distinction between the artery walls and lumen. The vessel walls and blood velocity were traced in B-mode frames via pixel density and frequency distribution algorithm. The software automatically calculated the relative diameter change, time to peak (following cuff release) and shear rate area-under-the-curve (SR_{AUC}). The peak artery FMD was defined as the peak percentage change in artery diameter from baseline to during 3-minutes post cuff release. Although the initial region of interest selection was operator-determined, the remaining analysis was independent of operator bias. FMD data was analysed with covariate control for baseline artery diameter (adjusted FMD) allowing FMD to be scaled for changes in artery diameter [45].

Statistical analysis

All data were analyzed using SPSS (Version 25.0, SPSS, Chicago, IL) and G-Power (version 3.1). A univariate general linear model was used to analyze the differences between i.) PRE-M and POST-M women; ii.) a sub-group of 20 pre- (N=10) and post-menopausal women (N=10) that were menopausal for <5 years in line with previous work [4]. A Pearson correlation was performed to statistically examine relations between vascular parameters and potential confounders (body fat, $\dot{V}O_{2max}$, PA and sedentary behaviour). Accordingly, $\dot{V}O_{2max}$ was the only confounder significantly related to vascular parameters and was subsequently entered as a co-variate into the general linear model. There were no statistically significant correlations apparent in the secondary analysis. Women in the secondary analysis were identified as the

10 oldest pre-menopausal women (Late-PRE-M), and women who were menopausal for <5 years (Early-POST-M). This analysis acknowledges the challenge of differentiating between the relative contributions from two parallel and intimately linked processes i.e. age and menopause and attempts to eliminate the role of age on the vascular measures as significant age-related vascular changes are likely to be limited within this short time frame. This study achieved >80% power at an α of 0.05 to find between-group differences in our main outcome of FMD with an effect size of 0.62 when comparing PRE- and POST-M women. Post-hoc power analysis also indicates that in our sub-group, sixteen women are required in each group in order to detect a difference of 2.5% in FMD. As such, the data generated from the secondary analysis is preliminary and will inform future studies measuring systemic vascular parameters around the time of menopause. Data are presented as mean [95% confidence intervals]. Data in tables and figures are presented as mean (SD). Statistical significance was assumed at $p<0.05$.

Results

Participant Characteristics

Women were POST-M for 6.5±4.3 years based on the time from their last menstrual period. POST-M women had a higher SBP (126 [123, 130 mmHg] $p<0.001$) and DBP (72 [70, 74 mmHg] $p=0.002$) compared to PRE-M women (Table 1). POST-M women had a higher body fat (34 [31, 36 %] $p=0.09$) compared to PRE-M (31 [28, 34%] $p=0.09$) however this did not reach statistical significance. Body mass and BMI were similar between PRE-M and POST-M women ($p=0.26$ and 0.87 respectively).

In the secondary analysis, the time since the last menstrual cycle for Early-POST-M women was 2.6±1.3 years (Table 1). The Late-PRE-M were significantly younger (45.9 [44, 48 years]) than the Early-POST-M group (50.4 [48, 52 years] $p=0.003$) but there were no differences in body mass ($p=0.48$), BMI ($p=0.63$), body fat ($p=0.49$), SBP ($p=0.85$) or DBP ($p=0.75$) between the groups.

Physical activity, sedentary behaviour and cardiorespiratory fitness

POST-M women had a significantly lower $\dot{V}O_{2max}$ (24.8 [22.6, 26.9 ml·kg·min]) compared to PRE-M (34.7 [32.6, 36.9 ml·kg·min] p<0.001). Daily vigorous PA was significantly lower for POST-M women (5 [3, 7 min/d]) compared to PRE-M women (9 [7, 11 min/d] p=0.01). POST-M women had a lower amount of sedentary time (490 [461, 520 min/d] p=0.09) compared to PRE-M women (525 [497, 553 min/d] p=0.09) however this did not reach statistical significance. There were no differences between groups for light (p=0.40), moderate PA (p=0.24), average daily PA (p=0.81) or accelerometer wear time (p=0.64, Table 1). Women in the Late-PRE-M group performed a greater amount of light PA (354 [292, 415 min/d]) than the Early-POST-M group (264 [199, 329 min/d] p=0.05). There were no differences between groups for $\dot{V}O_{2max}$, moderate PA (p=0.25), vigorous PA (p=0.92), average daily PA (p=0.12) or sedentary behaviour (p=0.11). Late-PRE-M women had significantly greater accelerometer wear time (858 [785, 930 min/d]) compared to Early-POST-M women (743 [616, 819 min/d] p=0.04).

Cerebrovascular Measurements

POST-M women had a significantly lower baseline MCAv (61.3 [57, 66 cm/s] p=0.03) and CVC (0.53 [0.45, 0.66 $cm \cdot s^{-1} \cdot mmHg^{-1}$] p=0.02) compared to PRE-M women (Table 2). There were no differences between PRE-M and POST-M for $PETCO_2$ (p=0.99), absolute (p=0.52) and relative MCA CVR (p=0.18), r^2 value (p=0.55), or CCA diameter response to the CVR test (p=0.64). In the secondary analysis comparing Late-PRE-M and Early-POST-M, there were no differences in baseline MCAv (p=0.91), CVC (p=0.07), $PETCO_2$ (p=0.23) absolute (p=0.79) and relative CVR (p=0.99), r^2 value (p=0.53), or carotid artery diameter response to the CVR test (p=0.25). There were no differences between PRE-M and POST-M women for the cerebral autoregulation parameters of normalised gain (p=0.56) and phase (p=0.73) measured in the low frequency (Table 2). Similarly, there were no differences between Late-PRE-M and Early-POST-M women for normalised gain (p=0.77) and phase (p=0.18) measured in the low frequency (Table 3).

Carotid artery reactivity

CAR was significantly lower in the POST-M women (0.7 [-0.7, 2.1%)] compared to PRE-M women (3.0 [1.5, 4.4 %] p=0.03) (Table 3). In the secondary analysis, CAR was similar between Early-POST-M (0.81 [-2.94, 4.56 %]) and Late-PRE-M (0.77 [-2.98, 4.51 %] p=0.99) (Table 3, Figure 1).

Peripheral Vascular Function

Brachial Femoral FMD and PWV

The POST-M women had a significantly lower brachial FMD (4.1 [2.9, 5.2 %]) compared to PRE-M women (6.4 [5.4, 7.5%] p=0.004) (Figure 2). The time to peak (TTP) was significantly higher in POST-M women (65 [57, 74 seconds]) compared to PRE-M women (46 [39, 54 seconds] p=0.002). There were no differences between groups for baseline diameter (Dbase) (p=0.62), peak diameter (Dpeak) (p=0.75), or shear rate area under the curve (SR_{AUC}) (p=0.10, Table 3). POST-M women had a significantly lower femoral FMD (2.8% [1.9, 3.6 %]) compared to PRE-M women (5.8 [4.9, 6.7 %] p<0.001) (Figure 2). There were no differences between PRE- and POST-M groups for femoral TTP (p=0.15), Dbase (p=0.19), Dpeak (p=0.72), or SR_{AUC} (p=0.76) (Table 3). PWV was significantly higher for POST-M women (6.87 [6.5, 7.3 m/s]) compare to PRE-M women (5.45 [5.1, 5.8 m/s] p<0.001). However, when $\dot{V}O_{2max}$ was controlled for, this was not statistically significant (p=0.49).

In the secondary analysis, brachial TTP was higher for Late-PRE-M women (66 [50, 82 seconds]) compared to the Early-POST-M group (37 [30, 49 seconds] p=0.01). There were no significant differences between Late-PRE-M and Early-POST-M women for bFMD (p=0.58), SR_{AUC} (p=0.09), Dbase (p=0.73), or Dpeak (p=0.53). Early-POST-M women had a significantly lower fFMD (2.1% [0.8, 3.5 %]) compared to Late-PRE-M women (4.1 [2.7, 5.5 %] p=0.049) (Figure 3). There were no differences between groups for TTP (p=0.69), Dbase (p=0.80), Dpeak (p=0.86) or SR_{AUC} (p=0.99). Allometric scaling did not alter the FMD

responses reported for brachial or femoral arteries (Table 3). PWV was not different between Early-POST-M and Late-PRE-M (p=0.84, Table 3).

Carotid and Peripheral Vascular Structure

Intima Media Thickness

POST-M had a greater IMT at the carotid (0.70 [0.68, 0.73 mm] p<0.001), brachial (0.38 [0.36, 0.41mm] p=0.004) and femoral arteries (0.49 [0.46, 0.53 mm] p=0.04) compared to PRE-M women (Figure 3, Table 3). POST-M women also had a higher IMT-to-lumen ratio at the carotid (0.10 [0.10, 0.11] p<0.001), brachial (0.10 [0.09, 0.11] p=0.001) and femoral arteries (0.06 [0.07, 0.08] p<0.001) compared to PRE-M women. Carotid artery diameter was greater in POST-M women (6.85 [6.7, 6.9 mm]) compared to PRE-M women (6.6 [6.5, 7.7 mm] p=0.014). However, when $\dot{V}O_{2max}$ was controlled for, this was no longer statistically significant (p=0.46).

Early-POST-M women had a significantly larger cIMT (0.67 [0.63, 0.63 mm]) compared to Late-PRE-M women (0.59 [0.54, 0.65 mm] p=0.03). There was a trend for a larger brachial IMT in the Early-POST-M group (0.34 mm [0.30, 0.37 mm]) compared to the Late-PRE-M group (0.30 [0.27, 0.33 mm] p=0.09). There were no differences between groups for femoral IMT (p=0.43) (Table 3). Early-POST-M women had a significantly greater carotid IMT/lumen (0.10 [0.09, 0.11]) compared to Late-PRE-M women (0.89 [0.09, 0.10] p<0.001). A trend was observed towards a larger brachial IMT/lumen artery in the Early-POST-M group (0.09 [0.08, 0.10]) compared to the Late-PRE-M group (0.08 [0.06, 0.09] p=0.05). There were no differences between groups for femoral IMT/lumen (p=0.62). There were no differences between groups for carotid artery diameter (p=0.71). Brachial and femoral artery diameters are referred to in the FMD section.

Discussion

This study aimed to describe the peripheral and cerebrovascular structural and function differences between PRE-M and POST-M women with simultaneous assessment of PA and

CRF. The findings of this study demonstrate that cerebral blood flow, central and peripheral vascular function are lower and artery wall thickness is greater in menopausal women. These changes occur with simultaneous reductions in vigorous PA and CRF. The secondary analysis performed to generate preliminary data around the time of menopause suggests that only femoral FMD and carotid IMT are impaired in early menopausal (<5 years). This proposes potential direct effects of oestrogen reduction and not age on large artery structure and function however, this warrants further investigation for improved clarification. Taken together, our data illustrates that menopause is accompanied by impaired MCAv, peripheral vascular function and structure with some site-specific decrements potentially evident early in the menopausal transition.

Cerebral blood flow, measured via MCAv, was lower in PRE-M compared to POST-M however, this was not apparent in the early years of menopause suggesting that this lower MCAv is likely age-related [9]. Nevertheless, CVR did not differ between PRE-M to POST-M, or in the secondary group analysis; in line with previous findings [16]. Reduced CVR is more commonly associated with populations with overt disease [46,47] and is usually maintained in healthy individuals [48] however, the immediate and long term effect of menopause on CVR without the inclusion of hormone therapy [13] has been largely unreported. Given the exclusion criteria of the current study (i.e. women on medication or with a history of disease) our findings suggest that cerebrovascular function is maintained during and following the menopause in healthy women with no CVD risk factors or overt CVD. In contrast to a previous study, cerebral autoregulation did not differ between PRE-M to POST-M, or in the secondary group analysis in contrast to a previous study [9]. This may be explained by differential methods used to assess cerebral autoregulation with ours adhering to recommended guidelines for assessing cerebral autoregulation [35]. In summary, cerebrovascular function appears unaltered with menopause despite reductions in MCAv.

A novel aspect of the current study relates to the assessment of CAR via the cold pressor test. This was included given the role of the carotid artery as an extra cranial vessel delivering

blood flow to the brain [49]. In addition, the CAR test reflects coronary artery dilatory responses [38] which may be indicative of coronary artery disease and CV events [50]. Thus, taken together CAR may provide insight into the interaction between central and cerebral vessels as well as central and peripheral vessels. Our data illustrates CAR to be lower in POST-M compared to PRE-M however, could potentially be age related since the CAR response was similar between Late-PRE-M and Early-POST-M women. Further clarification is required with a larger sample around the time of menopause to confirm this. Interestingly, given the extracranial role of the carotid artery, the CAR data does not reflect the intact in-tact CVR and autoregulatory responses between PRE-M and POST-M women. It does however support the age-related reductions in MCAv as well as peripheral artery vascular function (brachial and femoral arteries) observed in the current study. The dilation (or constriction) during the CAR test is influenced by sympathetic nerve activity [51], which also increases with age [52]. This age-associated increase in sympathetic nerve activity may explain the lower CAR in POST-M women. Alternatively, the diminished response at the carotid artery may also reflect endothelial dysfunction in line with that observed at the brachial artery as a consequence of age. We also included an assessment of femoral artery endothelial function in the current study to obtain a systemic view of the vascular system with age and menopause in females but also because, like the carotid artery, the femoral artery is susceptible to atherosclerosis [53]. Importantly, we show femoral artery endothelial dysfunction to be evident early in the menopausal transition according to our secondary analysis. While this may imply that femoral artery dysfunction may be due to menopause transition rather than age, caution is advised when interpreting these results given the small sample size involved. Nonetheless, we can speculate on the potential mechanisms regarding this. Firstly, this could be explained by differences in the contribution of oestrogen to vasodilation in the femoral artery via nitric oxide or oestrogen receptors located in the endothelial and smooth muscle cells [54]. Alternatively, the impairment could be explained by the reduction in PA observed in the current study. It is known that artery size influences the shear stimuli and functional responses of an artery [55]. An acute prolonged sitting intervention (i.e. reduction in PA) is associated a reduction in femoral but not

brachial FMD [56]. It is plausible that chronic reductions in PA could exacerbate this response further resulting in longer-term femoral artery dysfunction, however this requires further clarification. Taken together, the CAR test reflects lower systemic endothelial function in POST-M women that is potentially age related. In contrast, lower femoral artery endothelial function in POST-M women may be driven by reduced oestrogen and/or physical inactivity, however this requires further investigation. Our data supports the concept of site-specific arterial adaptation highlighting the need to assess multiple vascular sites in order to gain a comprehensive understanding of systemic haemodynamics.

Artery wall thickening at the carotid, femoral and brachial arteries were evident in POST-M compared with PRE-M women. Previous research has shown that significant carotid artery remodelling occurs post-menopause independent of atherosclerotic risk factors and metabolic variables [57]. Our preliminary data generated by the secondary analysis supports this, suggesting that remodelling may occur early in menopause and is potentially more pronounced at the carotid compared with brachial and femoral arteries. Increased BP, which exerts a greater distending force on the artery wall is considered an important contributor to carotid thickening [58] but elevated BP was not apparent in the early-menopausal period in this study. The carotid artery is susceptible to atherosclerosis due to its large artery diameter and the greatest shear rate in comparison to the brachial and femoral arteries [59]. Although speculative, the higher susceptibility of carotid artery wall thickening in early menopause may therefore be related to the elastic nature of the artery wall [60]. Oestrogen has the ability to increase elastin/collagen ratio and attenuate collagen deposition in aortic smooth muscle cells in vitro [61,62]. Oestrogen reduction may therefore have an opposing effect on the connective tissue component of the carotid artery wall leading to increased stiffness and altered structure [63]. In contrast, brachial and femoral arteries are muscular in nature with thicker medial layers and more smooth muscle cells compared with elastic arteries [64]. Increases in IMT in healthy adults are thought to be due to smooth muscle cell hypertrophy within the medial layer, and as such, muscular arteries may have more plasticity [65]. This may explain the prevalence of

carotid artery wall thickening compared to the periphery however this requires further investigation within a larger cohort around the time of menopause. According to previous research, maintaining moderate-vigorous PA in early menopause may influence several putative factors known to modulate smooth muscle cells in the arterial wall including sympathetic-adrenergic activity, circulating ANG II and endothelin-1, and locally released vasoactive factors such as nitric oxide [66-68]. It would therefore be reasonable to suggest that a reduction in this moderate-vigorous PA could compound these vasoactive factors. Interestingly, POST-M had a lower level of vigorous PA and simultaneously, a higher brachial and femoral IMT in comparison to PRE-M women. Previous findings have shown exercise to protect against IMT development in ageing men and women [69]. It is understood that exercise protects against IMT development by releasing local vasoactive factors at the endothelium to counteract vasoconstrictors associated with artery stiffening and wall thickening [66]. Acute increases in blood flow and a resulting elevation in shear stress [70] is known to increase nitric oxide production [71]. The withdrawal of this stimulus may therefore contribute to IMT development in the periphery. Taken together, the heterogenous progression of IMT across multiple arteries may be explained by differential artery wall properties and the influence of PA in attenuating peripheral IMT development. Overall these data imply for the first time, that menopausal status may potentially play a role in the regulation of carotid artery structure and femoral artery function. Remaining outcomes such as CAR, brachial and femoral structures and brachial FMD appear to be more heavily influenced by age. Nonetheless, further investigation into the time just before and soon after menopause (<5years) would clarify the contribution of age and/or oestrogen reduction on these parameters of central and peripheral vascular function and structure. This may influence future study designs protocols where age and/or menopause are being investigated.

This study, for the first time, examined important confounders of CVD including body composition, aerobic capacity, PA and sedentary behaviour in conjunction with a large battery of CVD risk markers in order to gain a more complete understanding of the influencers of CVD

risk in women. Despite 84% of women in this cohort surpassing PA guidelines of 150 min/week of moderate to vigorous PA, CRF were lower in PRE-M compared to POST-M women. This may be due to the significant reduction in vigorous PA in POST-M women, as vigorous PA has been shown to significantly impact CRF. Consequently, in our cohort of healthy women, achieving PA guidelines was insufficient to prevent against the presence of CV risk markers, in agreement with existing research [72]. Future research should look to identify a dose response to vigorous PA and CV risk factors among older women in an attempt to preserve confounders of CVD.

Strengths and Limitations

Firstly, this study is strengthened by a large sample size and the inclusion of a vast range of peripheral vascular and cerebrovascular outcomes that are novel risk factors for CVD compared to traditional use of fitness and BMI. These techniques have been combined with other traditional confounders of CVD risk including body composition, aerobic capacity, objectively measured PA and sedentary behaviour that have been typically assessed in isolation in this cohort. The wide age range has facilitated two separate analysis to be performed providing novel insight between PRE-M and POST-M women and Late-PRE-M and Early-POST-M women. For the first time in this cohort, we have applied allometric scaling to the brachial and femoral arteries during our FMD analyses to account for changes in baseline diameter.

A key limitation is our cross-sectional study design which limits the ability to monitor changes to CV risk parameters over time. As such the interpretation of our findings is limited as we cannot definitively determine the CV changes that arise with the ageing process. We acknowledge that no metabolic data was collected in this sample including blood glucose and lipid profiles which are important contributors to prevalence of CVD. Furthermore, the authors did not collect data on the number of women who expressed interest in the study and as such, are unable to comment on potential bias of the recruited sample. Additionally, 84% of the cohort included in this study met PA guidelines of 150 minutes moderate to vigorous PA per

week which may not be representative of the general female population and may have influenced our findings. In the secondary analysis, the authors did not exclude women form the late-pre-menopausal group based on vasomotor symptoms and as a result cannot confirm if some of these women were peri-menopausal. Importantly, the results of this secondary analysis must be interpreted with caution based on the sample size however, we were able to show significant statistical differences. Furthermore, we were unable to age match the Early-Post-M and Late-PRE-M groups, as such we have been unable to wholly remove the influence of age in this comparison.

Conclusion

Female vascular health is multifactorial and cannot be linked solely to oestrogen reduction. Our findings demonstrate that cerebral blood flow and peripheral artery function reduce with age in healthy women. Furthermore, oestrogen reduction appears to result in an immediate lowering of femoral artery function and higher carotid artery wall thickness, which could be early targets for intervention during the menopausal period to prevent accelerated vascular dysfunction and heightened cardiovascular disease risk.

Acknowledgements

None.

References

1. Santos CY, Snyder PJ, Wu WC, Zhang M, Echeverria A, Alber J. Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: A review and synthesis. *Alzheimers Dement (Amst).* 2017;7:69-87.
2. de Kat AC, Dam V, Onland-Moret NC, Eijkemans MJ, Broekmans FJ, van der Schouw YT. Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. *BMC Med.* Jan 4 2017;15(1):2.
3. Gliemann L, Hellsten Y. The exercise timing hypothesis - Can exercise training compensate for the reduction in blood vessel function after menopause if timed right? *The Journal of Physiology.*0(ja).
4. Moreau KL, Hildreth KL, Meditz AL, Deane KD, Kohrt WM. Endothelial function is impaired across the stages of the menopause transition in healthy women. *J Clin Endocrinol Metab.* Dec 2012;97(12):4692-4700.
5. Beam CR, Kaneshiro C, Jang JY, Reynolds CA, Pedersen NL, Gatz M. Differences Between Women and Men in Incidence Rates of Dementia and Alzheimer's Disease. *J Alzheimers Dis.* 2018;64(4):1077-1083.
6. Li R, Singh M. Sex differences in cognitive impairment and Alzheimer's disease. *Front Neuroendocrinol.* Aug 2014;35(3):385-403.
7. Wong RHX, Evans HM, Howe PRC. Poor cerebrovascular function is an early marker of cognitive decline in healthy postmenopausal women. *Alzheimers Dement (N Y).* Sep 2016;2(3):162-168.
8. Davey DA. Prevention of Alzheimer's disease, cerebrovascular disease and dementia in women: the case for menopause hormone therapy. *Neurodegener Dis Manag.* Feb 2017;7(1):85-94.
9. Ainslie PN, Cotter JD, George KP, et al. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *J Physiol.* Aug 15 2008;586(16):4005-4010.
10. Leeuwis AE, Smith LA, Melbourne A, et al. Cerebral Blood Flow and Cognitive Functioning in a Community-Based, Multi-Ethnic Cohort: The SABRE Study. *Frontiers in aging neuroscience.* 2018;10:279.
11. Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol (1985).* Oct 2006;101(4):1252-1261.
12. Matteis M, Troisi E, Monaldo BC, Caltagirone C, Silvestrini M. Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke.* May 1998;29(5):963-967.
13. Penotti M, Sironi L, Amicarelli F, Flenda F, Gabrielli L, Vignali M. Surgically induced menopause and blood flow in cerebral arteries. *Fertil Steril.* May 2002;77(5):1086-1087.
14. Kurji A, Debert CT, Whitelaw WA, Rawling JM, Frayne R, Poulin MJ. Differences between middle cerebral artery blood velocity waveforms of young and postmenopausal women. *Menopause.* Mar-Apr 2006;13(2):303-313.
15. Kastrup A, Dichgans J, Niemeier M, Schabet M. Changes of cerebrovascular CO2 reactivity during normal aging. *Stroke.* Jul 1998;29(7):1311-1314.
16. Mitsis GD, Debert CT, Hajo MI, Marmarelis VZ, Poulin MJ. Nonlinear, multiple-input modeling of cerebral hemodynamics during baseline and hypercapnia in young and post-menopausal women. *Conf Proc IEEE Eng Med Biol Soc.* 2007;2007:2855-2858.
17. Deegan BM, Sorond FA, Galica A, Lipsitz LA, O'Laighin G, Serrador JM. Elderly women regulate brain blood flow better than men do. *Stroke.* Jul 2011;42(7):1988-1993.
18. Black MA, Cable NT, Thijssen DH, Green DJ. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. *Am J Physiol Heart Circ Physiol.* Sep 2009;297(3):H1109-1116.
19. Swift DL, Earnest CP, Blair SN, Church TS. The effect of different doses of aerobic exercise training on endothelial function in postmenopausal women with elevated blood pressure: results from the DREW study. *British Journal of Sports Medicine.* 2011;46(10):753-758.

20. Bailey TG, Cable NT, Aziz N, et al. Exercise training reduces the acute physiological severity of post-menopausal hot flushes. *J Physiol.* Feb 1 2016;594(3):657-667.

21. Leslie MS, Briggs LA. Preeclampsia and the Risk of Future Vascular Disease and Mortality: A Review. *Journal of midwifery & women's health.* May 2016;61(3):315-324.

22. Liu SH, Wang JJ, Cheng DC, Su CH, Lin TH. Assessment of the endothelial function with changed volume of brachial artery by menstrual cycle. *Biomed Eng Online.* Sep 06 2016;15(1):106.

23. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med.* 1970;2(2):92-98.

24. Billat V, Dhonneur G, Mille-Hamard L, et al. Case Studies in Physiology: Maximal oxygen consumption and performance in a centenarian cyclist. *J Appl Physiol (1985).* Mar 1 2017;122(3):430-434.

25. Choi L, Liu Z, Matthews CE, Buchowski MS. Validation of accelerometer wear and nonwear time classification algorithm. *Med Sci Sports Exerc.* Feb 2011;43(2):357-364.

26. Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments in field-based research. *Med Sci Sports Exerc.* Nov 2005;37(11 Suppl):S531-543.

27. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. *J Sci Med Sport.* Sep 2011;14(5):411-416.

28. Healy GN, Wijndaele K, Dunstan DW, et al. Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). *Diabetes Care.* Feb 2008;31(2):369-371.

29. Willie CK, Colino FL, Bailey DM, et al. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods.* Mar 30 2011;196(2):221-237.

30. Ainslie PN, Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol.* May 2009;296(5):R1473-1495.

31. Giller CA, Hatab MR, Giller AM. Estimation of vessel flow and diameter during cerebral vasospasm using transcranial Doppler indices. *Neurosurgery.* May 1998;42(5):1076-1081; discussion 1081-1072.

32. Skow RJ, MacKay CM, Tymko MM, et al. Differential cerebrovascular CO(2) reactivity in anterior and posterior cerebral circulations. *Respir Physiol Neurobiol.* Oct 1 2013;189(1):76-86.

33. Carter SE, Draijer R, Holder SM, Brown L, Thijssen DHJ, Hopkins ND. Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. *Journal of Applied Physiology.* 2018;125(3):790-798.

34. Thomas KN, Lewis NC, Hill BG, Ainslie PN. Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. *Am J Physiol Regul Integr Comp Physiol.* Oct 2015;309(7):R707-720.

35. Claassen JA, Meel-van den Abeelen AS, Simpson DM, Panerai RB, international Cerebral Autoregulation Research N. Transfer function analysis of dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation Research Network. *J Cereb Blood Flow Metab.* Apr 2016;36(4):665-680.

36. Brackley KJ, Ramsay MM, Broughton Pipkin F, Rubin PC. A longitudinal study of maternal bloodflow in normal pregnancy and the puerperium: analysis of Doppler waveforms using Laplace transform techniques. *Br J Obstet Gynaecol.* Jan 1998;105(1):68-77.

37. Triedman JK, Saul JP. Blood pressure modulation by central venous pressure and respiration. Buffering effects of the heart rate reflexes. *Circulation.* Jan 1994;89(1):169-179.

38. van Mil AC, Hartman Y, van Oorschot F, et al. Correlation of carotid artery reactivity with cardiovascular risk factors and coronary artery vasodilator responses in asymptomatic, healthy volunteers. *J Hypertens.* May 2017;35(5):1026-1034.

39. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB, Sr. Carotid-wall intima-media thickness and cardiovascular events. *N Engl J Med.* Jul 21 2011;365(3):213-221.

40. Bruno RM, Bianchini E, Faita F, Taddei S, Ghiadoni L. Intima media thickness, pulse wave velocity, and flow mediated dilation. *Cardiovasc Ultrasound.* Aug 23 2014;12:34.

41. Bianchini E, Bozec E, Gemignani V, et al. Assessment of carotid stiffness and intima-media thickness from ultrasound data: comparison between two methods. *J Ultrasound Med.* Aug 2010;29(8):1169-1175.

42. Thijssen DH, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol.* Jan 2011;300(1):H2-12.

43. Thijssen DHJ, Bruno RM, van Mil A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J.* Jun 18 2019.

44. Woodman RJ, Playford DA, Watts GF, et al. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol (1985).* Aug 2001;91(2):929-937.

45. Atkinson G, Batterham AM. The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vasc Med.* Dec 2013;18(6):354-365.

46. Strandgaard S, Paulson OB. Cerebral autoregulation. *Stroke.* May-Jun 1984;15(3):413-416.

47. Markus H, Cullinane M. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain.* 2001;124(3):457-467.

48. Schwertfeger N, Neu P, Schlattmann P, Lemke H, Heuser I, Bajbouj M. Cerebrovascular reactivity over time course in healthy subjects. *Journal of the neurological sciences.* Nov 15 2006;249(2):135-139.

49. Faraci FM, Heistad DD, Mayhan WG. Role of large arteries in regulation of blood flow to brain stem in cats. *The Journal of Physiology.* 1987;387(1):115-123.

50. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation.* Apr 25 2000;101(16):1899-1906.

51. Berkenboom G, Unger P. Alpha-adrenergic coronary constriction in effort angina. *Basic Res Cardiol.* 1990;85 Suppl 1:359-369.

52. Rehman HU, Masson EA. Neuroendocrinology of ageing. *Age Ageing.* Jul 2001;30(4):279-287.

53. Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ. Heterogeneity in conduit artery function in humans: impact of arterial size. *Am J Physiol Heart Circ Physiol.* Nov 2008;295(5):H1927-1934.

54. Moreau KL, Donato AJ, Tanaka H, Jones PP, Gates PE, Seals DR. Basal leg blood flow in healthy women is related to age and hormone replacement therapy status. *J Physiol.* Feb 15 2003;547(Pt 1):309-316.

55. Thijssen DH, Willems L, van den Munckhof I, et al. Impact of wall thickness on conduit artery function in humans: is there a "Folkow" effect? *Atherosclerosis.* Aug 2011;217(2):415-419.

56. Thosar SS, Bielko SL, Wiggins CC, Wallace JP. Differences in brachial and femoral artery responses to prolonged sitting. *Cardiovasc Ultrasound.* Dec 15 2014;12:50.

57. Muscelli E, Kozakova M, Flyvbjerg A, et al. The effect of menopause on carotid artery remodeling, insulin sensitivity, and plasma adiponectin in healthy women. *Am J Hypertens.* Apr 2009;22(4):364-370.

58. Tanaka H, Dinenno FA, Monahan KD, DeSouza CA, Seals DR. Carotid artery wall hypertrophy with age is related to local systolic blood pressure in healthy men. *Arterioscler Thromb Vasc Biol.* Jan 2001;21(1):82-87.

59. Wu SP, Ringgaard S, Oyre S, Hansen MS, Rasmus S, Pedersen EM. Wall shear rates differ between the normal carotid, femoral, and brachial arteries: an in vivo MRI study. *J Magn Reson Imaging.* Feb 2004;19(2):188-193.

60. Binder EF, Birge SJ, Kohrt WM. Effects of endurance exercise and hormone replacement therapy on serum lipids in older women. *J Am Geriatr Soc.* Mar 1996;44(3):231-236.
61. Chironi G, Gariepy J, Denarie N, et al. Influence of hypertension on early carotid artery remodeling. *Arterioscler Thromb Vasc Biol.* Aug 1 2003;23(8):1460-1464.
62. Natoli AK, Medley TL, Ahimastos AA, et al. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension.* Nov 2005;46(5):1129-1134.
63. Westendorp IC, in 't Veld BA, Bots ML, et al. Hormone replacement therapy and intima-media thickness of the common carotid artery: the Rotterdam study. *Stroke.* Dec 1999;30(12):2562-2567.
64. Sturek M, Mokelke E, R. Sindermann J, P. Adam L, March K. Molecular and Cellular Physiology of Differentiated Vascular Smooth Muscle. 2007:1511-1523.
65. Dinenno FA, Tanaka H, Monahan KD, et al. Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men. *J Physiol.* Jul 1 2001;534(Pt 1):287-295.
66. Kingwell BA. Nitric oxide as a metabolic regulator during exercise: effects of training in health and disease. *Clin Exp Pharmacol Physiol.* Apr 2000;27(4):239-250.
67. Espeland MA, Applegate W, Furberg CD, Lefkowitz D, Rice L, Hunninghake D. Estrogen replacement therapy and progression of intimal-medial thickness in the carotid arteries of postmenopausal women. ACAPS Investigators. Asymptomatic Carotid Atherosclerosis Progression Study. *Am J Epidemiol.* Nov 15 1995;142(10):1011-1019.
68. Majmudar NG, Robson SC, Ford GA. Effects of the menopause, gender, and estrogen replacement therapy on vascular nitric oxide activity. *J Clin Endocrinol Metab.* Apr 2000;85(4):1577-1583.
69. Moreau KL, Silver AE, Dinenno FA, Seals DR. Habitual aerobic exercise is associated with smaller femoral artery intima-media thickness with age in healthy men and women. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology.* Oct 2006;13(5):805-811.
70. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nature reviews. Endocrinology.* Nov 2012;8(11):639-649.
71. Buchanan TA, Xiang AH. Gestational diabetes mellitus. *J Clin Invest.* Mar 2005;115(3):485-491.
72. Akbartabartoori M, Lean ME, Hankey CR. The associations between current recommendation for physical activity and cardiovascular risks associated with obesity. *Eur J Clin Nutr.* Jan 2008;62(1):1-9.

TABLES LEGEND:

Table 1 Participant characteristics, physical activity and sedentary behaviour between PRE-M and POST-M women and Late-PRE-M and Early-POST-M women.

Table 2 Cerebral hemodynamic differences between PRE- and POST-M women and Late-PRE-M and Early-POST-M women.

Table 3 Vascular data between PRE- and POST-M women and Late-PRE-M and Early-POST-M women.

FIGURE LEGEND:

Figure 1: Carotid artery reactivity (CAR) for PRE-M and POST-M women (*A*) and women in the secondary analysis (*B*). CAR is significantly lower in POST-M women compared to PRE-M women (*A*). A greater number of POST-M women demonstrate a constriction of the carotid artery during the cold pressor test (*p=0.03). Carotid artery reactivity is unchanged in early menopause (Early-POST-M) compared to late pre-menopause (Late-PRE-M) (p>0.05) (*B*).

Figure 2: Brachial and femoral artery flow-mediated dilation (FMD) for PRE-M and POST-M women and Late-PRE-M and Early-POST-M women. Brachial artery FMD is significantly lower in POST-M compared to PRE-M women (*p=0.004) although is not different between Late-PRE-M and Early-POST-M women (p=0.58). Femoral FMD is significantly lower in POST-M compared to PRE-M women (†p<0.001) and similarly, is significantly lower in Early-POST-M compared to Late-PRE-M women (‡p=0.049).

Figure 3: Intima-media thickness (IMT) at the carotid, brachial (bIMT) and femoral (fIMT) arteries are significantly higher in PRE-M compared to POST-M women (*p<0.001, †p=0.001).

Table 1 Participant characteristics, physical activity and sedentary behaviour between PRE-M and POST-M women and Late-PRE-M and Early-POST-M women.

Characteristic	PRE-M	POST-M	p-value	Late-PRE-M	Early-POST-M	p-value
N	50	50	-	10	10	-
Time from menopause (y)	-	6.5±4.3	-	-	2.6 1.3	-
Age (y)	33.2±9.1	58.5±5.5	**<0.001**	45.9±3.1	50.4±2.8[c]	**0.003**
Body mass (kg)	68.6±1.9	65.4±1.9	0.25	68.1±6.3	65.8±8.1	0.48
BMI (kg/m^2)	25±6	25±4	0.87	25±3	26±3	0.63
Body fat (%)	31.2±8.6	34.1±8.0	0.09	33.6±5.9	35.2±5.0	0.49
$\dot{V}O_{2max}$ (ml·kg·min)	34.8±8.6	24.8±6.2[b]	**<0.001**	31.6±6.0	28.2±7.6	0.29
SBP (mmHg)[a]	109±8	126±15[b]	**<0.001**	114±10	115±13	0.85
DBP (mmHg)[a]	65±7	72±7[c]	**0.002**	69±8	70±8	0.75
Physical Activity and Sedentary Behaviour						
Light PA (mins)	285±74	299±88	0.40	354±80	264±104	**0.05**
% Wear Time	34±7	35±9	0.88	41±8	35±10	0.41
Moderate PA (min/d)	55±22	49±24	0.24	43±18	55±23	0.25
% Wear Time	7±3	6±3	0.94	5±2	8±4	0.38
Vigorous PA (min/d)	10±9	5±7[c]	**0.01**	9±8	9±8	0.92
% Wear Time	1±1	1±1	0.98	1±1	1±1	0.98
Average daily PA (min/d)	350±78	353±94	0.81	406±85	328±118	0.12
Average daily wear time (min/d)	832±140	846±135	0.64	858±101	743±118[c]	**0.04**
Average daily sedentary time (min/d)	524±80	490±100	0.09	519±55	445±108	0.11

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause, POST-M, post-menopause, Late-PRE-M; late pre-menopause, Early-POST-M; early post-menopause, BMI; body mass index, $\dot{V}O_{2max}$; maximal oxygen consumption, SBP; systolic blood pressure, DBP; diastolic blood pressure, PA; physical activity. [a]$\dot{V}O_{2max}$ treated as covariate, significance is denoted by [b]$p<0.001$ and [c]$p<0.05$.

Table 2 Cerebral hemodynamic differences between PRE-M and POST-M women and Late-PRE-M and Early-POST-M women.

	PRE-M	POST-M	p-value	Late-PRE-M	Early-POST-M	p-value
$PETCO_2$ (mmHg)	36.2±3.2	36.2±3.6	0.99	36.3±3.3	41.9 ± 13.6	0.23
MCAv (cm/s^{-1})[a]	72.0±14.9	61.3±15.4[c]	**0.03**	68.9±6.0	68.2±16.3	0.91
CVC ($cm \cdot s^{-1} \cdot mmHg^{-1}$)[a]	0.79±0.33	0.53±0.32[c]	**0.02**	0.82±0.12	0.56±0.41	**0.07**
MAP (mmHg)[a]	80±7	90±9[b]	**<0.001**	83±9	88±8	0.62
Hypercapnic CVR test						
$PETCO_2$ (mmHg)	44.9±2.1	44.5±3.5	0.97	45.1±2.1	45.0±3.0	0.99
Carotid Diameter (mm)	6.46±0.01	6.64±0.01	0.18	6.37±0.17	6.28±0.17	0.72
Carotid Diameter (mm) (last 30 seconds)	6.49±0.06	6.37±0.15	0.64	6.45±0.24	6.86±0.25	0.25
CVR (r^2)	0.82±0.08	0.81±0.09	0.55	0.83±0.07	0.80 ± 0.11	0.53
Absolute CVR ($cm \cdot s \cdot mmHg^{-1}$)	3.76±1.48	3.51±1.92	0.52	4.03±1.56	4.31 ±2.58	0.79
Relative CVR ($cm \cdot s \cdot mmHg^{-1}$)	4.84±1.99	5.47±2.17	0.18	5.43±2.51	5.45 ± 2.36	0.99
Autoregulation						
$PETCO_2$ (mmHg)	38.2±2.5	38.0±2.1	0.98	37.1 1.1	37.4 2.0	0.97
MAP (mmHg)	80±9	89±7	0.10	84±3	88±5	0.58
Normalised gain (%)	1.35±0.37	1.30±0.40	0.56	1.35±0.29	1.31±0.28	0.77
Gain ($cm \cdot s \cdot mmHg$)[a]	0.98±0.22	0.72±0.22[b]	**<0.001**	0.92±0.17	0.83±0.19	0.35
Phase (degrees)	22.61±14.77	23.79±13.22	0.73	24.92±12.65	14.74±11.87	0.18
Coherence	0.62±0.12	0.67±0.12	-	0.67±0.12	0.62±0.08	-

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause; POST-M; post-menopause; Late-PRE-M; late pre-menopause, Early-POST-M; early post-menopause, $PETCO_2$; end tidal carbon dioxide MCAv; middle cerebral artery velocity, CVC; cerebrovascular conductance, MAP; mean arterial pressure, CVR; cerebrovascular reactivity. [a]$\dot{V}O_{2max}$ treated as covariate, significance is denoted by [b]$p<0.001$ and [c]$p<0.05$.

Table 3 Vascular data between PRE-M and POST-M women and Late-PRE-M and Early-POST-M women.

		PRE-M (N=48)	POST-M (N=41)	p-value	Late-PRE-M (N=10)	Early-POST-M (N=10)	p-value
	Pulse-Wave Velocity (m/s)[a]	5.45±0.99	6.87±1.40	**0.49**	5.87 ± 0.73	5.79 ± 1.05	0.84
FMD	**Brachial Artery**						
	Baseline artery diameter (cm)	0.34±0.04	0.34±0.04	0.62	0.35±0.04	0.34±0.06	0.73
	Peak artery diameter (cm)	0.36±0.05	0.35±0.05	0.75	0.37±0.04	0.36±0.07	0.53
	Time to Peak (secs)	46±23	65±32[b]	**0.002**	37±17	66±27[b]	**0.01**
	SR_{AUC} ($x10^3$)	16.8±7.7	20.0±10.9	0.10	14.1±6.3	20.9±9.4	0.09
	FMD (%)	6.4±3.9	4.1±3.4[b]	**0.004**	5.3±2.6	4.7±1.2	0.58
	Adjusted FMD (%)	6.4±0.5	4.2±0.6[b]	**0.007**	6.1±1.3	4.0±0.7	0.30
IMT (N=64)	IMT (mm)[a]	0.31±0.04	0.38±0.08[b]	**0.004**	0.30±0.04	0.34±0.30	0.09
	IMT/Lumen	0.08±0.14	0.10±0.02[b]	**0.001**	0.08±0.02	0.09±0.01	**0.05**
FMD	**Femoral Artery**						
	Baseline artery diameter (cm)	0.58±0.08	0.60±0.09	0.19	0.60±0.06	0.62±0.05	0.80
	Peak artery diameter (cm)	0.61±0.08	0.62±0.09	0.72	0.61±0.06	0.63±0.05	0.86
	Time to Peak (secs)	60±33	71±36	0.15	63±48	71±39	0.69
	SR_{AUC} ($x10^3$)	20.3±16.1	19.3±13.2	0.76	17.3±8.8	17.3±11.2	0.99
	FMD (%)	5.8±4.1	2.8±2.3[c]	**<0.001**	4.1±1.9	2.1±1.9	**0.05**
	Adjusted FMD (%)	6.0±0.4	2.5±0.4[c]	**<0.001**	5.3±1.1	2.2±1.0	**0.05**
IMT	IMT (mm)[a]	0.39±0.09	0.49±0.11[b]	**0.04**	0.46±0.08	0.49±0.10	0.43
	IMT/Lumen	0.07±0.01	0.06±0.01[b]	**0.01**	0.08±0.02	0.08±0.02	0.62
Carotid Artery	Lumen artery diameter (mm)[a]	6.59±0.40	6.85±0.53	**0.46**	6.61±0.30	6.72±0.54	0.71
	IMT (mm)[a]	0.54±0.07	0.70±0.08[c]	**<0.001**	0.59±0.07	0.67±0.09[b]	**0.03**
	IMT/Lumen	0.08±0.01	0.10±0.01[c]	**<0.001**	0.09±0.01	0.10±0.01[b]	**0.04**
Reactivity	CAR (%)	3.0±4.6	0.7±4.9[b]	**0.03**	0.8±6.0	0.8±4.5	0.99

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause, POST-M; post-menopause, Late-PRE-M; late pre-menopause, Early-POST-M; early post-menopause, $SR_{AUC;}$ shear rate area under the curve, FMD; flow-mediated dilation, IMT; intima media thickness, IMT/lumen; IMT-to-lumen ratio, CAR; carotid artery reactivity. [a]$\dot{V}O_{2max}$ treated as covariate, significance is denoted by [b]$p<0.001$ and [c]$p<0.05$.

Figure 1

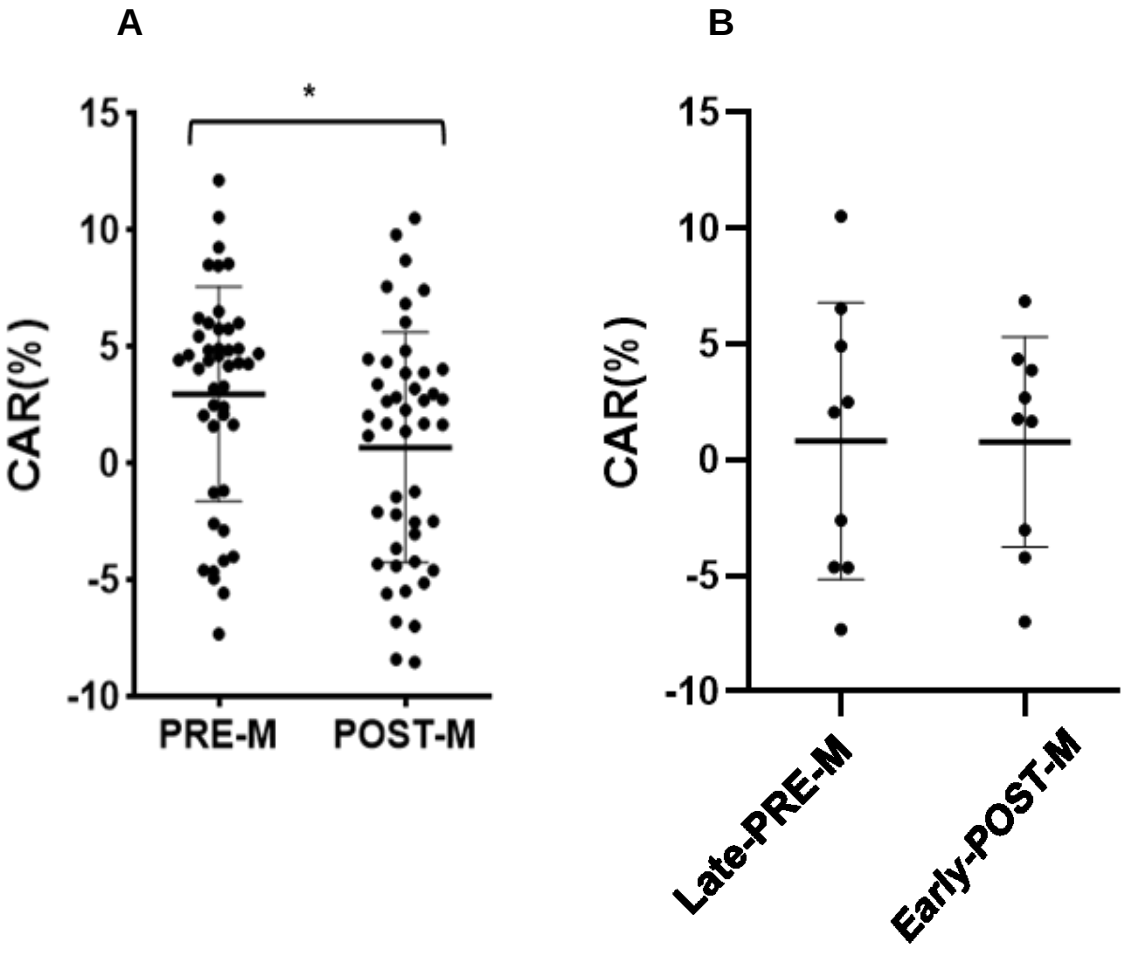


Figure 2

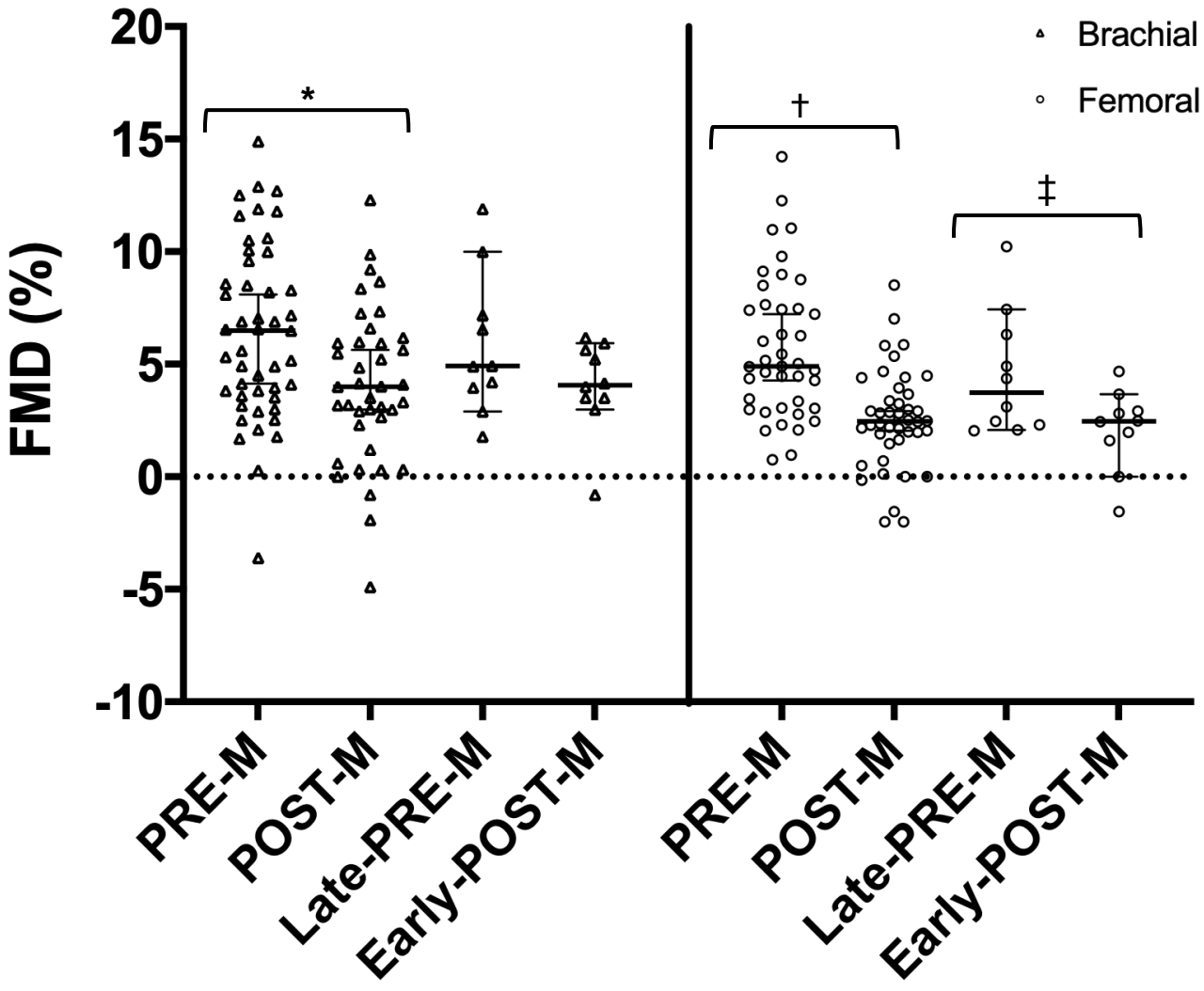
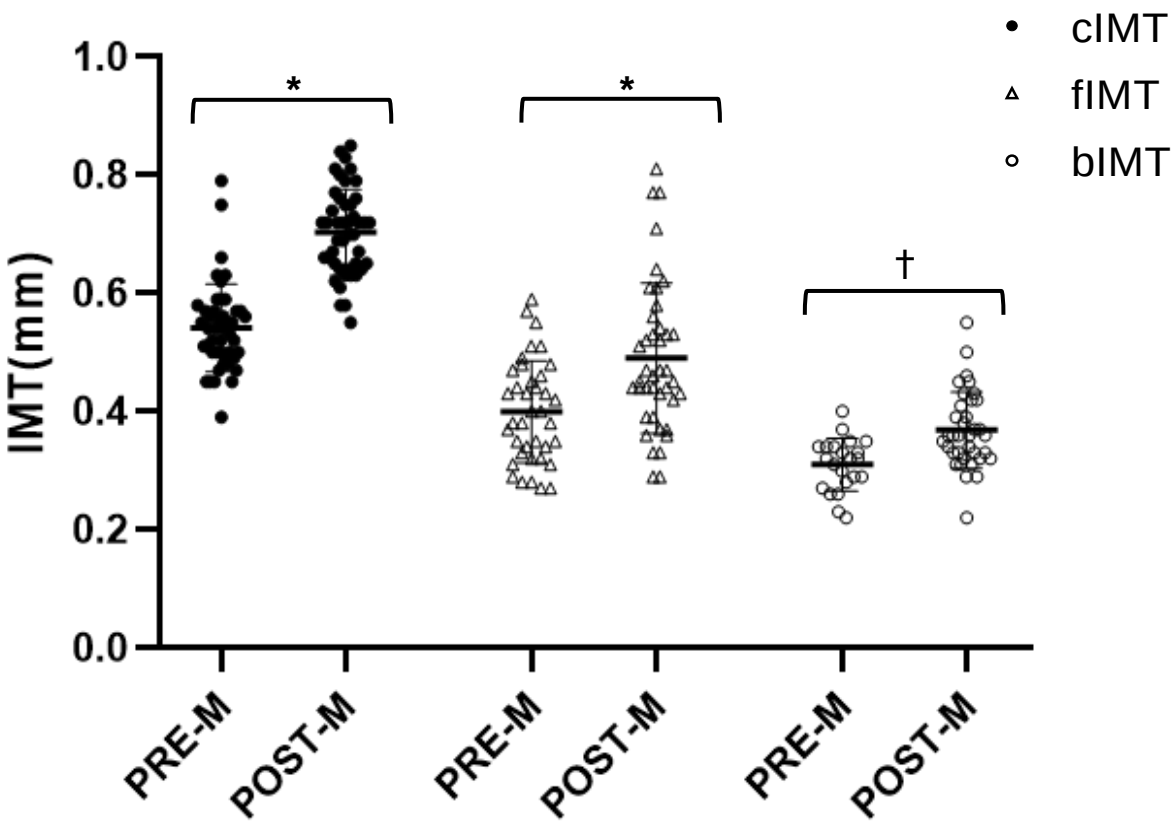

Brachial
Femoral
FMD (%)
20
15
10
5
0
-5
-10
*
†
‡
PRE-M
POST-M
Late-PRE-M
Early-POST-M
PRE-M
POST-M
Late-PRE-M
Early-POST-M

Figure 3


cIMT
fIMT
bIMT
IMT(mm)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
†
PRE-M
POST-M
PRE-M
POST-M
PRE-M
POST-M