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Title: The impact of uninterrupted sitting on central and peripheral cardiovascular function in pre-menopausal and post-menopausal women.

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Abstract:

Independently, both prolonged uninterrupted sitting and the onset of menopause negatively impact markers of cardiovascular risk. Whether their combination augment these responses additively remains unknown. This study assessed whether prolonged uninterrupted sitting causes greater central and peripheral cardiovascular dysfunction in post-menopausal women compared to pre-menopausal women. To address this, twenty-three healthy women (13 pre-menopausal [43.77 ± 4.30 years.] and 10 post-menopausal [57.20 ± 8.55 years.]) sat uninterrupted for 2-hours. Carotid-femoral pulse wave velocity (cf-PWV), pulse wave analysis (PWA), lower-limb venous pooling (HHb), and calf-circumference were assessed pre-and post-sitting using general linear mixed models, with age as a covariate. Changes in MAP over time (both between and within groups) was assessed using a two-way repeated-measures-ANOVA. There were no significant interactions for any outcome measures. However, for cf-PWV, there was a significant main effect of group (Δ = 0.854 ± 0.354 $m \cdot s^{-1}$; p =0.026, ηp^2 =0.707). For PWA, only heart rate (HR) and pressure forwards (Pf) showed significant main effects of time [Δ = 6 ± 1 bts-min-1, $p < 0.001$, $\eta p^2 = 0.861$] and group [Δ = 3.893 ± 1.450 mmHg, $p = 0.016$, $\eta p^2 = 0.271$] respectively. Both HHb (Δ = 2.737 ± 0.952, p = 0.009, $\eta p^2 = 0.742$) and calf-circumference (Δ = 0.812 ± 0.128 cm, $p < 0.001$, $\eta p^2 = 0.863$) significantly increased over time. While post-menopausal women demonstrated greater overall arterial stiffness (increased cf-PWV at baseline), there was no difference in cardiovascular response (central or peripheral) to 2-hours of prolonged sitting between the pre- and post-menopausal women.

Keywords: prolonged sitting, arterial stiffness, menopause, carotid-femoral pulse wave velocity

Summary Table

What is known about the topic?

1. Sedentary behaviour, specifically prolonged uninterrupted sitting (1.5-6 hours) has been associated with heightened cardiovascular disease (CVD) risk.
2. Prolonged uninterrupted sitting, which has been shown to impair vascular function in healthy populations could be worse in post-menopausal, who are already predisposed to an increased CVD risk.
3. Sedentary behaviours are likely common in post-menopausal women given that only 28 % of women aged 45-64 years meet physical activity guidelines.

What this study adds?

1. There is no effect of prolonged sitting (120-minutes) on central and peripheral cardiovascular function in pre- and post-menopausal women.
2. While prolonged sitting increased calf circumference, likely via the significantly increased lower limb venous pooling (HHb), this was not matched with increased arterial stiffness, or blood pressure.
3. While our study builds upon the current model of sitting induced arterial stiffness with similar venous pooling, the absence of change in cf-PWV suggests other unfathomed mechanisms may contribute to this dysfunction.

Introduction

Independent of physical activity status, sedentary behaviour, specifically prolonged sitting (1.5-6 hours), defined as activities consisting of < 1.5 metabolic equivalents in seated, reclined, or supine posture, is associated with heightened cardiovascular disease (CVD) risk and all-cause mortality[1,2]. Short periods of prolonged uninterrupted sitting have been shown to impair vascular function, including a significant decrease in brachial and popliteal artery flow-mediated dilation (FMD) and increased lower-limb venous pooling], in healthy populations[3,4]. It may be that this vascular dysfunction in response to uninterrupted sitting could be worse in populations who are predisposed to an increased CVD risk such as type-2 diabetics, hypertensives, and post-menopausal women[5,6]. Sedentary behaviours are likely common in post-menopausal women given that only 28 % of this population, who are aged 45-64 years, meet physical activity guidelines [7]. Understanding whether 2-hours of prolonged sitting deleteriously impacts vascular function in post-menopausal women to a greater extent than pre-menopausal women is important in order to develop efficacious public health guidance, given its detrimental association with vascular and cardiometabolic factors[8,6].

In recent meta-analyses, Paterson et al reported uninterrupted sitting, ranging from 1.5 to 6 hrs (modal sitting duration of 3-hrs), significantly decreased lower-limb endothelial function, (FMD weighted mean difference [WMD] = − 2.12%, 95% CI = − 2.66 to − 1.59), and increased systolic (WMD = 3.2 mmHg, 95% CI = 0.6 to 5.8)[9] and mean arterial blood pressures (MAP) (WMD = 3.3 mmHg, 95% CI = 2.2 to 4.4)[10]. Credeur et al demonstrated worsening arterial stiffness, which can be determined centrally (carotid to femoral [cf-PWV]) (pre-sit vs post-sit = + 0.40 $m \cdot s^{-1}$, $p = 0.009$, $d = 0.36$) in response to uninterrupted sitting[11]. Post-menopausal women may have a greater sitting-induced PWV dysfunction compared to pre-menopausal women, which could be in part explained by greater oxidative stress, inflammation, vascular smooth muscle tone[12,13], and a reduction in estradiol, nitric-oxide synthesis, and anti-oxidant secretion[14]. Whilst it is apparent that both the onset of menopause and prolonged uninterrupted sitting independently heighten known markers of CVD risk, what is not known is whether the combination of these two factors augment these responses. As such, the aim of the current study was to determine whether prolonged uninterrupted sitting causes greater central and peripheral cardiovascular dysfunction in post-menopausal women compared to pre-menopausal women.

Material and Methods:

Participants:

Twenty-five women (13 pre-menopausal, 2 peri-menopausal, and 10 post-menopausal) aged 36-65 years were recruited. Participants were non-smokers, non-pregnant, and had no history of postural hypotension, cardiovascular, cardiometabolic, or respiratory diseases. All women had: 1) fasted plasma glucose <5.5 mmol·l), 2) resting blood pressure (BP): <140/90 mmHg, 3) BMI: 18 – 30 kg/m^2, and 4) were not on any antihypertensive or lipid-lowering medications or hormonal replacement therapy (HRT).

Menopausal status was determined using a validated 'menopause health questionnaire'[15]. Pre-menopausal women were determined as having a consistent menstrual cycle for at least 3 months and not taking hormone-based contraception, whilst post-menopausal women reported 12 consecutive months of amenorrhoea without taking HRT. On completion of the questionnaire, two women were identified as peri-menopausal and were thus excluded (23 women were included in the final experiment). Institutional ethical approval which conformed to the standards of the journal was granted prior to recruitment and testing and the study was conducted in accordance with the Declaration of Helsinki Declaration of Helsinki (REC Approval Code: REC.22.127.1d). All participants gave written informed consent.

Study Design:

Using a repeated-measures between and within subjects' design, participants were stratified by menopause status. Each participant visited the laboratory twice, once for familiarization and consenting, and the second time for the experimental trial. The researchers and statistician processing the outcome data were blinded for analysis.

Experimental protocol

Data collection took place in an environmentally controlled laboratory (temperature: 22 ± 1°C, relative humidity: 51 ± 2%). For the experimental trial, participants arrived between 08:30 and 12:00, and were 6-hours fasted consuming only water, having refrained from caffeine for 12-hours, and strenuous physical activity for 24-hours[16]. Stature and mass were assessed, and the participants were asked to empty their bladders prior to laying quiet on the test bed (Plinth 2000, Plinth Medical, Suffolk, UK) in the supine position for 20-minutes. During this period, an oscillometric BP cuff (SphygmoCor Xcel, AtCor Medical, Sydney, Australia) was fitted over the left brachial artery. A thigh cuff was placed around the left superficial femoral artery (SFA) to determine the cf-PWV. On their right side, a continuous-

wave near-infrared spectroscopy (NIRS) device (Artinis Portalite, Artinis Medical Systems, BV Zetten, Netherlands) was placed on the most prominent area of the gastrocnemius muscle belly to determine changes in venous pooling in lower limb[17]. After 20-minutes, the pre-sit measurements of PWA and PWV were conducted in supine position, following which the participants were passively manoeuvred to a seated position for 120-minutes using an electronic three-way tilt table. At the end of the 120-minutes sitting protocol, participants were shifted to supine position again, following which all post-sitting vascular measures of PWA and PWV were repeated (Figure 1).

Experimental procedures

Pulse Wave Velocity

The SphygmoCor XCEL device simultaneously captured the proximal and distal arterial waveforms respectively through a tonometer and volume displacement cuff to record the pulse transit time (PTT). PWV was then calculated by dividing PTT by the arterial path length, or PWV distance (*D*). Specifically, for the cf-PWV: first, the tonometer was placed at the point of greatest pulsation on the left common carotid artery, while the oscillometric cuff was fitted around the left superficial femoral artery (SFA) to obtain the carotid-femoral PTT (cf-PTT), the time difference between the diastolic feet of proximal (carotid) and distal (femoral) pulse waveforms[18]. Then, the carotid-femoral *D* (cf-*D*) was estimated by subtracting the 'suprasternal notch to carotid' distance from the 'suprasternal notch to top of the femoral cuff' distance at the centre line of the leg[18]. The arterial path length (cf-d) was estimated using the subtraction method[18]. Thus, cf-PWV was calculated as cf-PWV = cf-*D* / cf-PTT. All PWV measures were assessed in triplicate as a minimum with at least two measures being between 0.3 $m \cdot s^{-1}$ of each other. The average of the closest two measures was used in all analysis.

Pulse Wave Analysis

The SphygmoCor XCEL was used to conduct PWA assessments pre- and post- 120-minutes of sitting. In brief, oscillometric pressure waveforms were assessed during a brachial cuff inflation lasting approximately 30-seconds, followed by a 10-second sub-diastolic recording, of which a corresponding aortic waveform is generated using a validated transfer function[19]. From sub-diastolic recording, central: systolic BP (cSBP), diastolic BP (cDBP), pulse pressure (cPP), augmentation index (AIx), augmentation index normalized to a heart rate of 75 bpm (AIx@75), forward aortic pressure (Pf), backward aortic pressure (Pb) and the Buckberg subendocardial viability ratio (SEVR) were derived. The Artery Task Force suggest central blood pressure may be influenced by respiration by 2-4 mmHg[20]. As such, all PWA assessments were conducted in triplicate as a minimum, and quadruplicate if

variability in cSBP was >4mmHg. For all PWA variables, the average of the closest two were used for all analyses. In accordance with Sharman et al., each PWA assessment was separated by a 1-minute period[20].

Venous Pooling

Changes in lower limb venous pooling (HHb) during the 2-hours sitting was estimated using a continuous wave-NIRS device (Artinis Portalite). The device was covered by a black box to prevent contamination of signals by ambient light[17]. The Portalite device has three light-emitting diodes positioned at 30 mm, 35 mm, and 40 mm from a single receiver, emitting near-infrared light at a constant intensity. The optode transmits light at wavelengths of 760 and 850 nm to determine the oxyhemoglobin (Hb) and deoxyhemoglobin (HHb) respectively. NIRS cannot distinguish between myoglobin and haemoglobin and as such the combination of both are referred to as haemoglobin from here on. For the pre- vs post-sitting analysis absolute NIRS values were used, and AUC was calculated as an index of blood pooling over the 120-minutes in seated position.

Sample Size

Based on previous work, the minimum within-subject detectable difference in cf-PWV means between two time points, was conservatively set at 0.36 $m \cdot s^{-1}$ with the SD at 0.50 $m \cdot s^{-111}$. Thus, using the effect size of 0.36 derived from the main effect of time i.e., change in cf-PWV between pre-and post-120 min of sitting, and the maximum chances of type 1 error set at 5 % and power set at 0.90, the approximate number of participants required using G*Power was 23[21].

Statistical Analysis:

Statistical analyses were performed using JAMOVI software (version 2.3.21, package GAMLj)[22], a graphical front end to the R programming language[23]. Raw data are presented as mean ± SD. A Shapiro–Wilk test was performed to assess the distribution of normality. Differences in participants' demographic and anthropometrics were analysed using independent-samples *t*-tests (pre-menopause vs post-menopause). cf-PWV, PWA, venous pooling (HHb), and calf-circumference and were analyzed with a Time (Pre-sit, Post-sit) by Group (Pre-menopause, Post-menopause) general linear model (GLM). Due to the pressure dependent relationship between BP and cf-PWV, and our insufficient power to simultaneously covariate for changes in both MAP and age, used age as a covariate and we determined any changes in MAP over time both between and within groups using a two-way repeated-measures-ANOVA. Raw data are presented as mean ± SD, whereas mixed model data is presented as mean difference (MD) with 95 % confidence intervals (95% CI). The alpha level of

significance is set at ≤ 0.05 for all analyses. Effect size is reported using partial eta squared (η_p^2) where < 0.01, 0.06, and ≥ 0.14 were considered small, moderate, and large respectively[24].

Results

Participants

Participant characteristics are presented in **Table 1**. Twenty-three women (13 pre-menopausal and 10 post-menopausal), who self-identified themselves as Caucasian (n=20), and Asian (n=3) were included in the final analyses. All participants successfully completed the experimental trial. NIRS data file was corrupted for one participant (post-menopausal), and carotid pulsations could not be detected on the neck of another participant (post-menopausal), making PWV data unmeasurable With the exception of age and MAP, no baseline characteristics differ between groups (**Table 1**).

----Insert Table 1 near here----

Carotid-femoral Pulse Wave Velocity (cf-PWV) and Pulse Wave Analysis (PWA)

For cf-PWV, there was no significant interaction or main effect of time; however, there was a significant increase in the main effect of group (Δ = 0.854 ± 0.354 $m \cdot s^{-1}$; p =0.026, ηp^2 =0.707). There were no significant Time by Group interactions for any of the PWA variables (**Table 2**; all p > 0.05). However, there was a significant increase in the time main effect for HR (Δ = 6 ± 1 $bts\text{-}min^{-1}$; p=0.016; η_p^2 = 0.271) and group main effect for Pf (Δ = 3.893 ± 1.450 mmHg; p = 0.016; η_p^2 = 0.271).

----Insert Table 2 near here----

Venous pooling (HHb), and calf circumference

As shown in **Table 3**, for venous pooling (HHb), there was no significant interaction or main effect of group; however, there was a significant main effect of time (Δ = 2.737 ± 0.952 µmol, p = 0.009, η_p^2 = 0.742). Further, HHb area-under-curve (AUC) was not significantly different between groups (pre-menopausal = 206 ± 39.4 vs post-menopausal = 201 ± 28.4 µmol; p = 0.716, d = 0.160). For calf circumference, there was a significant main effect of time (Δ = 0.812 ± 0.128 cm, p < 0.001, η_p^2 = 0.863), but there were no significant interaction or group effect.

----Insert Table 3 near here----

Discussion

The aim of this study is to determine whether prolonged uninterrupted sitting causes greater central and peripheral cardiovascular dysfunction in post-menopausal women compared to pre-menopausal women. Contrary to expected, our study suggests that 2-hours of prolonged uninterrupted sitting causes no acute adverse effects on central or peripheral vascular function in either group. While prolonged sitting increased calf circumference, likely via the significantly increased lower limb venous pooling, this was not matched with increased arterial stiffness, or blood pressure. This is an important finding given that previous research which has investigated the effects of prolonged uninterrupted sitting in male only, or mixed sex studies, and have speculated that the increased arterial stiffness observed was due to increased venous pooling causing a reduction in SV, which in turn leads to reduced aortic shear stress with subsequent endothelial dysfunction and increased arterial stiffness (cf-PWV).

Strengths and Limitations

To better contextualise the findings of this study, it is important to first highlight the strengths and limitations before detailing any explanations of the data. Strengths: 1) Our heterogeneous cohort varied in age and ethnicity. We recruited them with the rationale of being inclusive and providing a better representation of the population. However, future studies should explore; how much intra-individual variations (e.g., physical activity status, menstrual cycle phase, sex hormone concentrations) impact cardiovascular responses to prolonged sitting. 2) Women tend to gain weight at the onset of menopause; however, our samples are similarly overweighted to account for the confounding effect of weight on the CVD outcome variables. Limitations: 1) We did not age match our pre- and post-menopausal groups; consequently, unable to completely remove the influence of age in this comparison, however as age was added as the covariate in our statistical analysis, we have likely accounted for any observed effect. 2) We were not sufficiently powered to covariate for both age, and so, changes in MAP were determined using a two-way repeated-measures-ANOVA. 3) The duration of laboratory sitting bouts in prolonged sitting studies ranged from 1.5 to 6-hours, with >70% using a 3-hours bout. However, we measured cardiovascular responses following 2-hours bout, which means we could have missed capturing the largest effect of sitting; however, uninterrupted sitting for 2-hours is ecologically valid whereas 3-hours is not[36]. 4) While participants self-reported their menstrual history via a validated questionnaire, and as such, any misdiagnoses of menopausal status were unlikely, we cannot guarantee accuracy; however, this is a validated questionnaire and so this is unlikely [15]. In the future, sex hormones (estradiol) estimation via salivary steroid immunoassays is needed to confirm the precise menopausal status.

Comparison to literature

We did not observe increased cf-PWV following 2-hours of prolonged uninterrupted sitting in pre- or post-menopausal women. Previous studies typically report a significant increase of 0.3 – 0.6 $m \cdot s^{-1}$ in cf-PWV following 180-minutes of prolonged uninterrupted sitting [11,25,17,26]. While the current study observed no significant change in cf-PWV following 2-hours of prolonged uninterrupted sitting in either pre- or post-menopausal women (pre- vs post- sit Δ = – 0.103 ± 0.192 $m \cdot s^{-1}$) (**Table 2**), we can conceive several potential factors to explain these findings. First, many of the previous studies have evaluated the prolonged sitting response in males. However, when Credeur et al., completed a sub analysis of female participant, there was no change in cf-PWV (pre- vs post-sit Δ = 0.1 $m \cdot s^{-1}$)[27] similar to what we have observed in post-menopausal women (pre- vs post-sit Δ cf-PWV = 0.02 $m \cdot s^{-1}$) (**Table 2**). Second, a recent study from Paterson et al reported no significant change in cf-PWV (MD = 0.12 $m \cdot s^{-1}$, SE = 0.06) following 2-hours of sitting (similar to our 2-hours sitting duration)[39]. And although 2 hours of prolonged sitting is ecologically more valid as it reflects typical sitting times in the population, our observed response might be due to the difference in time period of sitting (2 vs >3 hours), indeed the majority of PWV studies have >3 hours seated duration[11,25,17,26]. Therefore, the contrasting findings is likely due to the different time periods used. In support of this, although we saw a significant increase in lower-limb blood pooling (MD = 4.18 µmol) our changes appear smaller than those reported by Fryer et al (MD = 6.29 µmol) when sitting last for three hours[30]. Third, it has been shown that physical activity is negatively associated with arterial stiffness in post-menopausal women (sedentary vs moderate activity Δ cf-PWV= 0.600 ± 0.090 $m \cdot s^{-1}$)[28], and our study did not assess physical activity status prior to each experimental trial, we cannot rule out that this factor did not play a part in the attenuated response in cf-PWV to prolonged sitting. Finally, it is difficult to distinguish the effects of aging and menopause on vascular dysfunction since they are closely related. It is believed that estrogen plays a role in inhibiting oxidative stress and suppressing the production of NO through its antioxidant effect[37, 38]; nevertheless, the exact role of estrogen in the complex relationship between menopause, aging, and vascular dysfunction remains unclear.

Further, we determined the measures of central cardiovascular function via PWA. Interestingly, akin to the cf-PWV, prolonged uninterrupted sitting did not alter PWA (except HR) in either pre- or post-menopausal women (**Table 2**). While limited evidence exists regarding how acute prolonged sitting affects central haemodynamics, prior work from our group using orthostatic stressors provides an alternative insight[33]. Head-up tilt testing, induces venous pooling which diminishes venous return and stroke volume (SV). The autonomic nervous system compensates for this diminution of SV by increasing HR and total peripheral resistance i.e., constricting resistance and capacitance vessels[34,29]. However, these physiological changes only aligned with our significant compensatory increase in HR during sitting (**Table 2**). We also demonstrated higher Pf in post-menopausal women compared with pre-menopausal women (**Table 2**). It could be that our elevated cf-PWV in post-menopausal women contributes to this higher Pf amplitude rather than cSBP and cPP, leading to increased transmission of potentially harmful large forward pressure waves into the periphery and microcirculation[35].

We observed a significant change in venous pooling (HHb) (**Table 3**) following prolonged sitting in the

post-menopausal group (MD = 4.18 µmol), which is similar to those observed by Kelsch et al.[29], and Fryer et al. [30] (MD = 4.5 µmol and MD = 6.29 µmol, respectively). Further, as a proxy of venous pooling, we assessed calf circumference in line with other previous studies who did (Fryer et al)[26] and did not (Credeur)[30] assess HHb. However, our findings of a significant change in calf circumference during prolonged sitting in the Post-M group (MD = 0.9 cm, p <0.001) (**Table 3**), are less, compared to Fryer

et al. (MD = 1.42 cm)[26] and Credeur et al. (MD = 2.0 cm)[31], who both used 180-minutes seated time vs, our 120-minutes. Additionally, our blunted cf-PWV responses, compared to previously reported studies with longer duration (180-minutes or more) may be a product of our shorter (120-minutes) sitting time, causing comparatively less lower-limb venous pooling, i.e. for 180-minutes of sitting venous pooling increase by (4.50 – 6.29 µmol)[29,30] and for 120-minutes (in this study) it increased by 4.18 µmol, leading to minimal aortic dysfunction. While our study builds upon the current model of sitting induced arterial stiffness with similar venous pooling[32], the absence of change in cf-PWV suggests other unknown mechanisms may contribute to this dysfunction.

Conclusion and Implications

Our data suggests that there is no effect of prolonged sitting (120-minutes) on central and peripheral cardiovascular function in pre- and post-menopausal women, with post-menopausal women having higher aortic stiffness (greater cf-PWV) at baseline. Addressing sitting duration can be of importance whilst exploring the effect of prolonged sitting in this population as sedentary behaviour not only increases the risk of chronic illness, but is also associated with worse menopausal symptoms. While our study builds upon the current model of sitting induced arterial stiffness with similar venous pooling, the absence of change in cf-PWV suggests other mechanisms may contribute to this dysfunction. An increase in oxidative stress and decreased nitric oxide (NO) bioavailability may contribute to accelerated vascular dysfunction in estrogen-deficient postmenopausal women (referred earlier). Thus, to better understand vascular changes in this population, measuring sex hormonal concentrations could aid in interpretation of hemodynamic data, owing to their potential of obscuring the true effect of vascular dysfunction posed by prolonged sitting.

Data Availability

The data contained within will be made available upon reasonable request.

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Work is the authors own, and no funding was required for any part of this study.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

AM, KS, LT, CP, and SF conceived and designed research; AM and NH performed experiments; AM analysed and interpreted data; AM drafted initial manuscript; All authors edited and subsequently approved final version.

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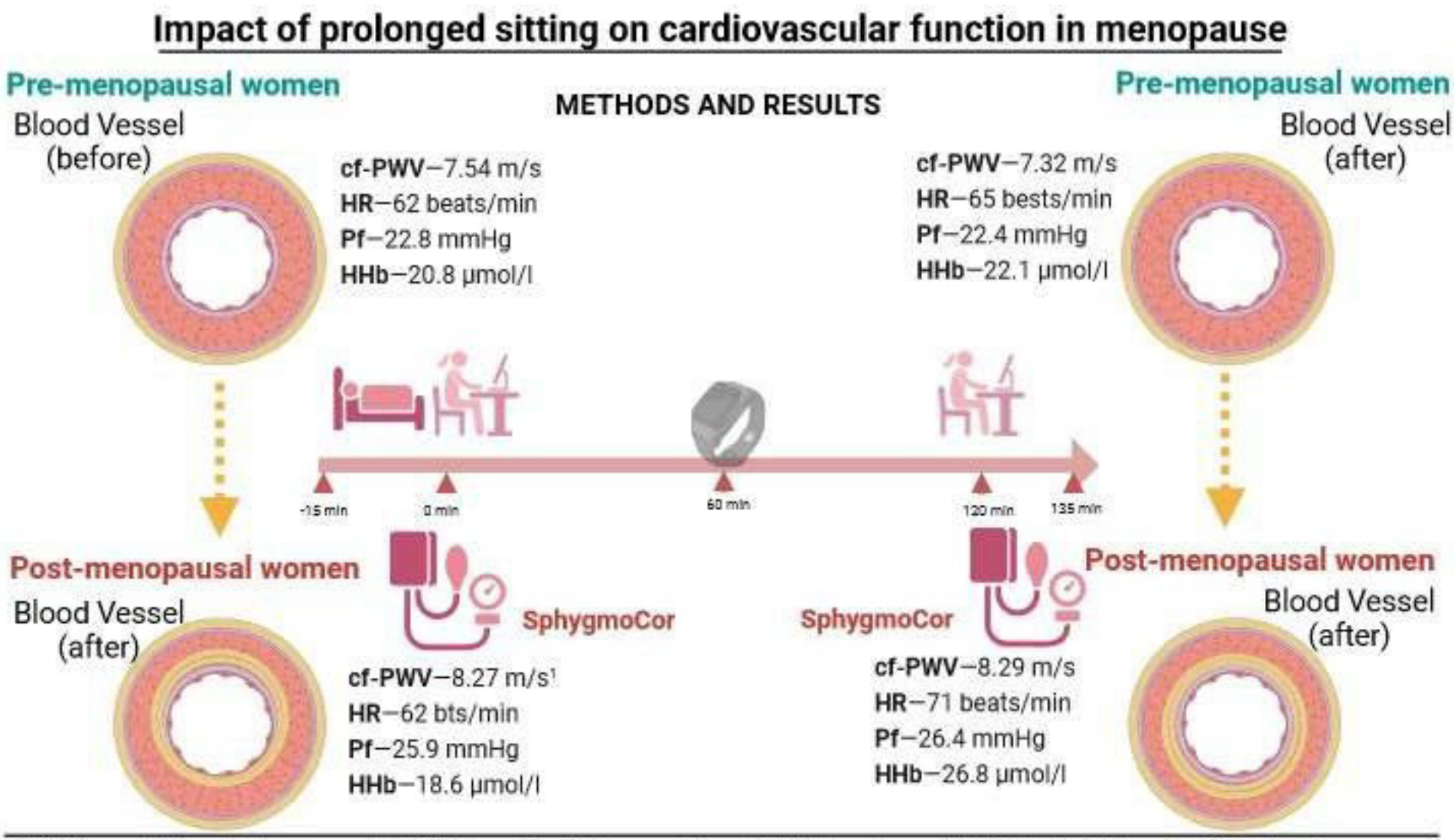

[a]cf-PWV: carotid-femoral pulse wave velocity; [a]HR: heart rate; [a]Pf: pressure forward; [a]HHb: deoxyhemoglobin; [a] Significant time effect; [b] Significant group effect

CONCLUSION: While post-menopausal women demonstrated greater overall arterial stiffness (increased cf-PWV at baseline), there was no difference in cardiovascular response to 2-hours of prolonged sitting between the pre- and post-menopausal women.

Table_1

Table 1. Participant characteristics for pre- (n=13) and menopausal (n=10) groups

	Pre-menopausal (n=13) Mean Average (SD)	Post-menopausal (n=10) Mean Average (SD)	*p*	Cohens' d
Age (years)	43.77 (4.30)	57.20 (8.55)	<0.001*	-2.074
Mass (kg)	73.48 (14.12)	71.19 (14.13)	0.704	0.162
Stature (m)	1.65 (0.10)	1.64 (0.05)	0.889	0.059
BMI (kg/m^2)	27.07 (4.72)	26.42 (5.32)	0.762	0.129
Blood Glucose ($mmol \cdot L^{-1}$)	3.98 (0.44)	3.98 (1.67)	0.997	-0.001
SBP (mmHg)	121 (10)	126 (23)	0.477	-0.304
DBP (mmHg)	75 (7)	78 (14)	0.580	-0.236
MAP (mmHg)	89 (7)	92 (16)	0.006*	-0.287

Data are presented as mean ± SD: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; mmHg: millimetres of mercury; m^2: meter square; p: significance; Cohens' d: effect size; * = $p < 0.05$

Table_2

Table 2 Mean (±SD), interaction, and main effects for cf-PWV and PWA pre (0 min) and post (120-minutes) prolonged sitting in pre- (n=13) and post-menopausal (n=10) groups

		cf-PWV ($m \cdot s^{-1}$)	Heart rate ($bts \cdot min^{-1}$)	SBP (mmHg)	MAP (mmHg)	c-SBP (mmHg)	DBP (mmHg)	c-PP (mmHg)	AIx (%)	AIx@75 (%)	Pf (mmHg)	Pb (mmHg)	SEVR (%)
Mean Average (SD)													
Pre-menopausal	0-minute	7.54	61.7	119	88	108.00	77	30.30	13.50	7.58	22.80	12.20	168.00
		(0.97)	(7.64)	(8)	(7)	(8.79)	(6)	(4.85)	(7.54)	(7.92)	(2.52)	(1.88)	(17.10)
	120-minutes	7.32	65.0	118	89	114.00	77	29.50	13.80	10.60	22.37	12.20	165.00
		(0.64)	(4.69)	(10)	(9)	(10.20)	(8)	(4.06)	(7.18)	(10.40)	(2.70)	(1.52)	(20.80)
Post-menopausal	0-minute	8.27	61.6	126	92	108.00	79	34.60	15.30	10.08	25.90	13.30	155.00
		(1.28)	(5.55)	(21)	(16)	(19.60)	(14)	(8.08)	(10.04)	(11.40)	(5.57)	(3.84)	(23.00)
	120-minutes	8.29	70.8	123	91	112.00	78	32.30	16.00	14.10	26.40	12.00	156.00
		(0.81)	(6.66)	(19)	(17)	(18.10)	(14)	(4.98)	(9.72)	(11.70)	(4.63)	(2.45)	(14.80)
Interaction Effect	*p*	0.542	0.796	0.362	0.554	0.183	0.612	0.570	0.955	0.977	0.723	0.148	0.632
	η_p^2	0.383	0.207	0.483	0.625	0.421	0.660	0.633	0.946	0.972	0.734	0.398	0.673
Time Effect	*p*	0.599	<0.001[a]	0.278	0.683	0.230	0.506	0.256	0.820	0.130	0.856	0.130	0.802
	η_p^2	0.349	0.861	0.527	0.707	0.890	0.596	0.460	0.813	0.388	0.844	0.387	0.798
Group Effect	*p*	0.026*	0.207	0.172	0.349	0.229	0.449	0.056	0.435	0.309	0.016[b]	0.091	0.105
	η_p^2	0.707	0.572	0.585	0.511	0.447	0.565	0.329	0.557	0.490	0.271	0.360	0.372

SD = standard deviation; *p* = significance; mmHg = pressure; $bts \cdot min^{-1}$ = beats per minute; mmHg= millimetre of mercury; SBP= systolic blood pressure; MAP= mean arterial pressure; c-SBP central systolic blood, DBP= diastolic blood pressure; c-PP = central pulse pressure AIx = augmentation index, AIx@75= augmentation index at HR of 75 $bts \cdot min^{-1}$; Pf= pressure forwards, Pb= pressure backwards; SEVR subendocardial variability ratio. *p* = significance; η_p^2 (partial Eta^2) = effect size; [a] Significant time effect; [b] Significant group effect.

Table_3

Table 3 Mean (± SD) interaction, and main effects for markers of lower limb blood pooling pre (0 min) and post (120-minutes) prolonged sitting in pre- (n=13) and post-menopausal (n=10) groups

		Venous Pooling (HHb) (µmol)	Calf circumference (cm)
Mean Average (SD)			
Pre-M	0-minute	20.80 (2.93)	38.50 (3.95)
	120-minutes	22.10 (4.93)	39.20 (4.09)
Post-M	0-minute	18.60 (1.97)	37.80 (4.06)
	120-minutes	22.80 (2.24)	38.70 (4.02)
Interaction Effect			
	p	0.148	0.496
	η_p^2	0.600	0.410
Time Effect			
	p	0.009[a]	<0.001[a]
	η_p^2	0.742	0.863
Group Effect			
	p	0.526	0.741
	η_p^2	0.392	0.252

Data are presented as mean ± SD; SD = standard deviation; *p* = significance; η_p^2 (partial Eta^2) = effect size; HHb = deoxyhaemoglobin; µmol = micromoles, [a] significant time effect.

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