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Title: The effect of cardiorespiratory fitness and habitual physical activity on cardiovascular responses to 2-hours of uninterrupted sitting

Running head: Physical activity, cardiorespiratory fitness, and cardiovascular responses to prolonged sitting

Type: Research Article

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Abstract

Prolonged uninterrupted sitting of >3-hours has been shown to acutely cause central and peripheral cardiovascular dysfunction. However, individuals rarely sit uninterrupted for >2-hours, and the cardiovascular response to this time is currently unknown. In addition, whilst increased cardiorespiratory fitness (CRF) and habitual physical activity (HPA) are independently associated with improvements in central and peripheral cardiovascular function, it remains unclear whether they influence the response to uninterrupted sitting. This study sought to 1) determine whether 2-hours of uninterrupted sitting acutely impairs carotid-femoral pulse wave velocity (cfPWV), femoral ankle PWV (faPWV), and central and peripheral blood pressure, and (2) to investigate the associations between CRF and HPA versus PWV changes during uninterrupted sitting. Following 2-hours of uninterrupted sitting, faPWV significantly increased (mean difference [MD] = 0.26 $m \cdot s^{-1}$, standard error [SE] = 0.10, p = 0.013) as did diastolic blood pressure (MD = 2.83 mmHg, SE = 1.08, p = 0.014), however cfPWV did not significantly change. Whilst our study shows 2-hours uninterrupted sitting significantly impairs faPWV, neither CRF (r = 0.105, p = 0.595) nor HPA (r = -0.228, p = 0.253) were associated with the increases.

New & Noteworthy We demonstrate that neither cardiorespiratory fitness nor habitual physical activity influence central and peripheral cardiovascular responses to a 2-hr bout of uninterrupted sitting in healthy young adults.

Keywords: Prolonged sitting; sedentary behaviour; arterial stiffness; cardiorespiratory fitness; physical activity

Introduction

Increased cardiorespiratory fitness (CRF) and habitual physical activity (HPA) are independently associated with a reduction in the prevalence and severity of multiple non-communicable diseases, of which the most common is cardiovascular disease (CVD) (1). Increasing CRF and HPA can facilitate improvements in the cardiovascular system, including, but not limited to, reduced central and peripheral blood pressures, reduced resting heart rate, increased stroke volume and cardiac output, and improved arterial stiffness (2–4). The purported principal physiological mechanism underpinning these enhancements is an improvement in endothelial and vascular function via increases in shear stress caused by being more active (2). Indeed, vascular function is imperative for the normal functioning of the cardiovascular system, with impairments in vascular function being an early marker of cardiovascular disease (5). Given the established health benefits, it may be that individuals with a higher CRF or those who undertake more HPA might have a certain level of cardiovascular protection against known risky lifestyle behaviours which heighten CVD and mortality risk. One such negative lifestyle behaviour is sedentarism, which predicts both incident CVD and mortality (6–8). The most common sedentary behaviour, and one which is known to impair cardiovascular function, is prolonged uninterrupted sitting (9–11).

Given the high prevalence of uninterrupted sitting in modernised economies (12–15) and its association with heightened CVD risk, several studies have sought to understand whether CRF can offset sitting-induced vascular dysfunction, but so far it has reached equivocal conclusions. Garten et al., (16) concluded that CRF had no impact on lower limb microvascular function (assessed using the passive leg movement technique) during 3 hours of uninterrupted sitting. Further, Morishima et al. (17) and Liu et al. (18) both investigated the influence of CRF on sitting-induced changes in popliteal artery flow-mediated dilation (FMD), a marker of vascular function, following 3 hours of uninterrupted sitting. Morishima et al. (17) found that FMD was maintained in individuals with a higher aerobic capacity following sitting (ΔFMD = -0.5 %, p = 0.196), whereas FMD significantly decreased in those with a lower aerobic capacity (ΔFMD = -2.7 %, p = 0.003). By contrast, Liu et al. (18) found a negative correlation between CRF and sitting-induced reduction in popliteal artery FMD following uninterrupted sitting (r = -0.51, p = 0.02), suggesting that those with a greater aerobic capacity may be acutely more susceptible to the deleterious effects of prolonged sitting. The reasons for these differences are unclear, however, it is clear that further work is needed to fully explore the effect of CRF on sitting-induced changes in vascular function. Further, to date, no research has investigated the associations between HPA and sitting-induced vascular dysfunction despite evidence to suggest that short-term reduction in HPA can impair lower limb vascular function (19) and that regular HPA can improve it (20).

It should be noted that whilst vascular function is commonly assessed using flow-mediated dilation, an alternative marker is pulse wave velocity. Pulse wave velocity assesses the time taken for the arterial

pulse wave to propagate from a proximal to a distal point within the arterial tree with a greater velocity representing increased stiffness (21). Whilst FMD is a useful marker of vascular function, which is commonly used within sitting research (10), PWV may offer alternative benefits. Changes in PWV are at least partially influenced by endothelial function, and thus reflect vascular and endothelial function, and have the benefit of being able to assess a greater portion of the arterial tree (22). Arguably, popliteal FMD is only indicative of the small region of the arterial tree being imaged and may not reflect broader changes within the lower limb vasculature. Alternatively, femoral-ankle (fa)PWV assesses a greater portion of the lower limb vasculature and may also offer insight into the interplay of central (aortic) and peripheral arterial stiffness (23). A further benefit of PWV assessments is that they facilitate the assessment of functional changes in vascular function in arteries that cannot readily be imaged, such as the aorta. Central (or aortic) stiffness is typically assessed using carotid-femoral (cf)PWV which is considered the non-invasive reference standard (24). Long-term increases in cfPWV (reflecting detrimental increases in stiffness) are associated with increased risk of CVD (25–29).

Whilst a biologically plausible model linking acute changes in sitting-induced cardiovascular dysfunction to long term cardiovascular disease risk has not been fully established, cfPWV has been posited as a useful intermediate outcome owing to its established causal links with overall health outcomes (30,31). To effectively inform and develop public health guidelines related to sedentary behaviour, it is necessary to; 1) use markers of cardiovascular health which are established intermediate outcomes that have causal links with overall health outcomes (32,33,30), such as peripheral blood pressure, and cfPWV (25–29), and 2) use uninterrupted sitting times which represent real-world behaviours (31). The most common uninterrupted sitting duration used in experimental studies is 3 hours (10,11,34), however, contemporary evidence suggests that individuals rarely engage in sitting bouts >2 hours (35) and that the most pronounced increases in peripheral blood pressure occur within the first two hours of uninterrupted sitting (34). Whilst the sitting duration used in an experimental study should be informed by the research question and a balance between internal and external validity, it is necessary to investigate whether shorter, more realistic sitting bouts elicit deleterious effects on markers of central and peripheral cardiovascular health. Consequently, this study had two primary objectives (1) to determine whether 2-hours of uninterrupted sitting elicits deleterious effects on markers of central and peripheral cardiovascular health, specifically cfPWV, femoral ankle PWV (faPWV), and central and peripheral blood pressure, and (2) to investigate the associations between CRF and HPA versus changes in markers of central and peripheral cardiovascular health during prolonged sitting.

Methods

Participants

Twenty-nine young (25 – 44 yrs) apparently healthy participants were recruited from a university population. Participants were non-smokers, free of any known illness or metabolic disorders, nor were any taking known vascular-acting medications. Institutional ethics approval was granted prior to recruitment and all participants provided written informed consent. As previous research has indicated that neither sex (36) nor menstrual cycle phase (37) influence responses to uninterrupted sitting, this study was not designed to investigate such distinctions, as such menstrual cycle phase of female participants was not considered during recruitment.

Study Design

Participants completed two laboratory visits. The first visit comprised consenting, anthropometric measurements (stature and mass), and familiarisation with all the equipment. After which participants completed a treadmill based $\dot{V}O_{2max}$ test to determine CRF. Following completion of the $\dot{V}O_{2max}$ test, participants were fitted with an activPal activity monitor facilitating measurements of HPA and sedentary behaviour 7-days immediately preceding their second laboratory visit, the sitting trial. Participants were instructed to maintain their usual physical activity level during this period. During the second laboratory visit, participants sat uninterrupted for 2-hours, and all cardiovascular outcomes were assessed pre and post.

Experimental Protocol

Data collection took place in an environmentally controlled laboratory (temperature: 22 ± 1°C, relative humidity: 51 ± 2%). For the experimental visit, participants arrived at 0830 following an overnight fast, consuming only water, and having refrained from caffeine for 12 hours. Participants were also asked to refrain from alcohol and strenuous exercise for 24-hours prior. Upon arrival, participants were asked to void their bladder before laying on the test bed in a supine position for 20 minutes. During this period participants were fitted with oscillometric blood pressure cuffs over the left brachial artery, left thigh, and left ankle for assessment of central and peripheral blood pressures, cfPWV, and faPWV. To estimate lower limb blood pooling, a continuous-wave near-infrared spectroscopy (NIRS) device was placed over the belly of the gastrocnemius on the participant's right leg (38).

Following 20 minutes of quiet supine rest, baseline measures of central and peripheral blood pressure, and cfPWV and faPWV were collected using the SphygmoCor XCEL device (AtCor Medical, Australia). All measures of central and peripheral blood pressures and velocities were collected in triplicate and the average of the closest two was used in analyses. Following the completion of baseline measures, participants were moved to an adjacent comfortable sitting chair with a pillow placed underneath their feet to avoid passive muscle contractions. In the seated position, participants' hips and knees both maintained ~90° flexion throughout the sitting bout. Once seated, calf circumference was measured at the point of greatest girth on the participants' right leg and marked for post-sitting measures. Participants then sat uninterrupted for 2-hours. Whilst participants were permitted to use the

bathroom during this period, no participants did so. During the sitting bout, participants were reminded to avoid vigorous lower leg movement and not to cross their legs. A member of the research team monitored participant movement throughout the sitting bout to ensure adherence. Participants were allowed to conduct low stimulus activities such as work on a laptop or read a book and were allowed to consume water ad libitum. Following 2-hours of sitting, post measures of calf circumference were taken before participants were manoeuvred back to a supine position on the test bed. Following 20 minutes of supine rest, all pre-sitting cardiovascular measures were repeated in the same order.

Experimental Procedures

Habitual Physical Activity Monitoring

For the purposes of this study, HPA is characterised as daily step counts, as well as time spent in light (LPA) and moderate-to-vigorous physical activity (MVPA). Participants wore an activPal monitor (activPAL4, Pal Technologies Ltd, Glasgow, United Kingdom) sampling at 20 Hz for 24-h·day^{-1} concurrently for 7-days immediately prior to the prolonged sitting visit. To avoid any potential confounding, during the data collection period, the $\dot{V}O_{2max}$ was performed at least 8-10 days before the sitting visit. Additionally, owing to the pre-experimental visit instructions to avoid strenuous activity before the experimental visit, activity data from the day immediately prior to experimental visit was excluded from analysis. In line with manufacturers guidelines, the activPal was waterproofed and secured to the midline of the right thigh, one-third of the way between the hip and the knee using transparent waterproof dressing (Tegaderm, 3M). In line with previous recommendations, participants were provided with a standardised diary to report daily wake and sleep times and to report any instances of the device being removed during the data collection period (39). For a day to be considered valid for inclusion in analysis, wear time must have exceeded 80% of waking hours (determined using the participant diary) or, if waking hours were not recorded, a minimum wear time of 10 hours per day was required. A minimum of 5 valid days of data (including 1 weekend day) was needed to be considered valid (40). Data were initially analysed using PALanalysis software (Pal Technologies Ltd, Glasgow, United Kingdom) to determine daily step counts and time spent in sedentary behaviours before subsequent analysis in R (Version 4.2.2) (41) to calculate time spent in different physical activity intensities using previously validated step rate thresholds (42).

Cardiorespiratory Fitness Test

Participants completed a ramp-style maximal exercise test on a motorised treadmill (Pro XL, Woodway Inc., Waukesha, USA). $\dot{V}O_{2max}$ was measured using an online breath-by-breath system (Cortex Metamax 3B, Leipzig, Germany) which was calibrated prior to each testing session. All testing took place in an environmentally controlled chamber maintained at a constant temperature and humidity (18 °C and 50 % relative humidity, respectively). A ramp protocol was utilised as such protocols have been shown to be better tolerated by broader demographics, including those with lower exercise capacities

(43–45). The protocol consisted of a 2-min warm-up performed at 6 $km \cdot h^{-1}$ and a 1 % incline before gradually increasing speed at a rate of 1 $km \cdot h^{-1}$ every minute until volitional exhaustion or participants met the two following criteria (1) an apparent plateau in $\dot{V}O_2$ despite an increase in treadmill speed, and (2) a respiratory exchange ratio $\geq$ 1.10 (46).

Primary Outcomes: Pulse Wave Analysis and Regional Pulse Wave Velocity

Pulse wave analysis (PWA) and regional PWV (cfPWV and faPWV) were obtained using SphygmoCor XCEL (AtCor, Sydney, Australia). For PWA, oscillometric pressure waveforms were recorded at the brachial artery as well as peripheral blood pressure. A corresponding aortic pressure waveform was generated using a validated transfer function from which central blood pressure was estimated (47). Additionally, the generated aortic waveform was analysed to assess augmentation index (AIx), forward aortic pressure (Pf), and backward aortic pressure (Pb). All PWA assessments were conducted in triplicate as a minimum, and quadruplicate if variability in systolic blood pressure exceeded 4 mmHg. Each PWA assessment was separated by a 1-min period and the average of the closest two measures was used for all analyses.

Pulse wave velocity was determined by dividing arterial path length by pulse transit time (PTT). PTT was assessed by simultaneously capturing arterial pressure waveforms at proximal and distal sites using applanation tonometry and oscillometric cuffs, respectively. For cfPWV, the tonometer was placed on the left carotid artery at the point of greatest pulsation and the oscillometric cuff was placed on the left thigh at the level of the femoral artery, following manufacturer guidelines. Arterial path length was estimated using the subtraction method in line with current guidelines, whereby the distance between suprasternal notch and point of greatest carotid pulsation was subtracted from the distance between suprasternal notch and the top of the femoral oscillometric cuff (48). For faPWV, the tonometer was placed at the point of greatest pulsation on the left superficial femoral artery, whilst an oscillometric cuff was positioned immediately proximal to the left medial malleolus. Arterial path length was estimated by measuring the linear distance between the point of greatest pulsation and the top of the ankle cuff. Femoral-ankle PTT was then corrected as previously described prior to calculation of PWV (49,50).

Near-Infrared Spectroscopy

In line with previous studies, venous pooling was estimated using cw-NIRS to determine blood volume change in the gastrocnemius (38,51,52). The Artinis Portalite device is a single wired optode consisting of three light-emitting diodes positioned 30, 35, and 40 mm from a single receiver. The light emitting diodes transmit near-infrared light at wavelengths of 760 and 850 nm and allows the determination of oxy- (Hb) and deoxyhaemoglobin (HHb), the sum of which is total haemoglobin (tHb). An appropriate path length correction factor, specific to the calf, was employed to account for the scattering and absorption of light within the tissue. Signals were allowed to stabilise in the final 10-mins of supine

rest. Values were normalised to baseline in each trial rather than being reported as the absolute change. Positioning of the NIRS device was confirmed via ultrasound (Terrason T3300, Burlington, MA, USA).

Sample Size

No previous study has sought to investigate the relationship between CRF and HPA versus sitting induced changes in PWV, consequently, power analysis was conducted using data from previous literature using popliteal artery FMD. Using the observed correlation of $r = 0.51$ reported by Liu et al., (18) an alpha level of 0.05, and a power of 0.8, power analysis estimated that 22 participants would be required (53). The target sample was inflated to 30 to account for attrition and unknown sources of error.

Statistical Analysis

Statistical analyses were performed using Jamovi (Version 2.3.18) (54), and R (Version 4.2.2) (41). Raw data are presented as mean (standard deviation [SD]). Data were checked for normality using Shapiro-Wilk test and visual inspection of histograms and were found to be normally distributed. To answer Objective (1), paired samples *t*-tests were used for all pre-post assessments (PWA, PWV, HHb, tHb). Results are presented as mean difference (MD) and standard error (SE). Cohen's *d* was used as a measure of effect with < 0.2, 0.2, 0.5, and 0.8 representing a trivial, small, moderate, and large effect, respectively (55).

To answer Objective (2), partial Pearson correlation analyses were performed to determine the relationships between CRF (relative $\dot{V}O_{2max}$) and HPA (daily step counts, time spent in LPA and MVPA) versus sitting-induced changes (post-sitting value minus pre-sitting value) in measures of cfPWV and faPWV. Owing to the interdependence of PWV and blood pressure, all partial Pearson correlation analyses controlled for changes in mean arterial pressure (MAP) using the *ppcor* package (56). Due to the number of statistical comparisons, the Benjamini-Hochberg method was utilised to control the false discovery rate (57). Critical values for statistical significance were calculated separately for tests of difference (Objective 1) and tests of association (Objective 2). All *p* values presented hereafter have been corrected using the Benjamini-Hochberg method, with values ≤ 0.05 deemed statistically significant.

Results

Participants

Participant characteristics, CRF, HPA and sedentary behaviours are presented in **Table 1**. Thirty self-identified Caucasian individuals (23 males, 7 females) were recruited and enrolled in the current study, however, one participant failed to complete the prolonged sitting condition. Consequently, 29 Caucasian individuals (22 males, 7 females) completed both study visits. All data from the participant who failed to complete both visits have not been presented nor analysed.

Stimulus: Prolonged Sitting

Results for supine measures of PWA and segmental measures of PWV are presented in **Table 2**. Results from the primary outcomes, cfPWV and faPWV are presented in **Figure 1A** and **1B**, respectively. There were significant increases in faPWV (MD = 0.26 $m \cdot s^{-1}$, SE = 0.10, p = 0.037, d = 0.51) (**Figure 1B**), diastolic blood pressure (MD = 2.83 mmHg, SE = 1.08, p = 0.037, d = 0.49), central diastolic blood pressure (MD = 2.45 mmHg, SE = 0.99, p = 0.037, d = 0.47), and HHb (MD = 1.92 µmol, SE = 0.57, p = 0.011, d = 0.65). Additionally, there were significant decreases in AIx (MD = -7.09 %, SE = 1.03, p = 0.011, d = 1.28), AIx@75 (MD = -7.47 %, SE = 1.36, p = 0.011, d = 1.28), and Pb (MD = -1.09 mmHg, SE = 0.26, p = 0.013, d = 0.78). Seated calf circumference also significantly increased (pre: 38.5 cm (SD = 2.4) vs post: 39.6 (SD = 2.5), MD = 1.03 cm, SE = 0.097, p = 0.011, d = 1.96).

Associations Between Cardiorespiratory Fitness and Habitual Physical Activity Versus Change in Central and Peripheral Measures

Results from partial Pearson correlation analyses are presented in **Table 3**. Owing to equipment failure, HPA data was not available for one participant. Consequently, partial correlation analyses including HPA as a variable represent n = 28 participants whereas analyses including CRF as a variable represent n = 29. There were no significant associations between any metric of HPA and changes in cfPWV or faPWV. Additionally, there were no significant associations between CRF and changes in cfPWV or faPWV.

Discussion

This study had two primary objectives; (1) to determine whether 2-hours of uninterrupted sitting elicits deleterious effects on markers of central and peripheral cardiovascular health, specifically cfPWV, faPWV, and central and peripheral blood pressure, and (2) to investigate the associations between CRF and HPA versus the aforementioned markers of central and peripheral cardiovascular health. This study found that 2-hours of uninterrupted sitting resulted in significant increases in faPWV and other markers of cardiovascular health, however, the changes were not associated with either CRF or any metric of HPA. As such, these results suggest that neither CRF nor HPA impact central and peripheral blood pressure or arterial stiffness responses to 2-hours uninterrupted sitting in healthy adults.

Strengths and Limitations

To fully contextualise the findings of this study, it is first important to acknowledge the strengths and potential limitations. Firstly, despite efforts to recruit participants with a broad range of CRF levels, the current sample represents an above average level of aerobic capacity based on normative values for age

and sex (58). Indeed, the lowest $\dot{V}O_{2max}$ of any participant was 39 mL·kg^{-1}·min^{-1} which, according to normative data for age and sex, remains in the 50th percentile and is considered average (58). As such, it is still unclear how individuals with a below average $\dot{V}O_{2max}$ may influence the central and peripheral vascular response to uninterrupted sitting. Further work is needed to fully explore the relationship between CRF and cardiovascular responses to prolonged uninterrupted sitting and thus improve generalisability. Secondly, whilst the power calculation for this study was based on previous research following a similar design and sample size (n = 21) (18), the aforementioned study investigated popliteal artery FMD rather than central and peripheral measures of cardiovascular health. As such, it is still possible that there may be an effect of CRF or HPA on central and peripheral measures, but it may be smaller, thus a larger sample would be required to detect it. Thirdly, whilst every attempt was made to balance the study sample, it is predominantly comprised of male participants (75 % male, 25 % female) and menstrual cycle phase was not considered. However, previous research suggests that sex (36), menstrual cycle, and oral contraceptive pill phases (37) do not influence sitting-induced changes in popliteal FMD. Finally, it should be acknowledged that whilst this paper sought to investigate a more ecologically valid uninterrupted sitting bout of 2 hours, the restrictions placed on participants' lower leg movement still limit broader generalisability.

Comparison to Literature: 2-hours of Uninterrupted Sitting

This study showed that 2-hours of uninterrupted sitting resulted in significant increases in faPWV, and several markers of central and peripheral cardiovascular health. However, compared to the available literature, the non-significant change in cfPWV observed in the present study (MD = 0.12 m·$^{s-1}$, SE = 0.06) is in contrast to previous studies, which have shown increases ranging from 0.3 (51) to 0.5 (59) m·s^{-1}. This may be attributed to the shorter (1-hour less) sitting time producing a less pronounced detrimental stimulus to the vascular system. Sitting-induced increases in PWV are thought to be a result of negative autonomic, hormonal, metabolic, and haemodynamic responses (31). One of the key contributing haemodynamic mechanisms behind sitting-induced increases in PWV is thought to be venous pooling in the lower limb (31,60). Purportedly, venous pooling in the lower limbs reduces venous return, which in turn, reduces stroke volume. This reduction in stroke volume is hypothesised to reduce shear stress against the wall of the aorta, promoting acute oxidative stress and endothelial dysfunction, presenting as increased cfPWV (22). Indeed, in the current study, venous pooling (changes in HHb) (MD = 1.92 µmol, SE = 0.57, p = 0.011, d = 0.65) are notably less than Kelsch et al., (61) and Fryer et al., (62) who both used a 3-hour sitting period (MD = 4.5 µmol, 95 % CI: -1.64 to 10.6, p = 0.08, d = 0.55 and MD = 6.29 µmol, 95 % CI: 3.37 to 9.21, p < 0.001, d = 1.20 respectively). In addition, changes in calf circumference were also less in our study compared to Fryer et al., (62) (MD = 1.42 cm, 95% CI: 0.94 to 1.89) and Credeur et al. (63) (MD = 2.0 cm) who both used 3-hours sitting. Other contributing factors to the observed increases in PWV may be stimulation of the renin-angiotensin-aldosterone system as a result of reduced renal perfusion pressure elevating blood pressure, and with it,

arterial stiffness, as well as increased sympathetic nervous system activity and increased systemic vascular resistance. Whilst these additional mechanisms were not assessed in the current study, it is again conceivable that the shorter sitting bout may have resulted in a diminished stimulus.

Given the associations of lower limb PWV with CVD risk (23,64), the increase in faPWV observed in this study is an important one. It is likely that the increase in faPWV shares similar mechanisms to decreases in lower limb artery FMD that have been previously reported (10). Owing to the high concentration of vascular smooth muscle cells present in peripheral artery walls, whose function is mediated by endothelium-derived vasodilators (65), peripheral arteries may be more prone to detriments in endothelial function and may present as increased arterial stiffness (22). During a bout of uninterrupted sitting, reduced blood flow in the lower limbs, and therefore shear stress (66), coupled with increased hydrostatic pressure (67) and arterial bending (68) leads to decreased vasodilator availability and therefore impaired endothelial function. The increase in faPWV observed in the current study is lower than previously reported findings from studies with longer sitting durations but may again be a product of a shorter sitting time leading to less dysfunction. Given the interaction between central and peripheral arteries and the effect on central haemodynamics, understanding the effect of prolonged sitting on lower limb arterial stiffness is an important step. Pressure wave reflection sites occur at bifurcations and points at which arterial geometry changes and contribute to normal function. However, in the presence of increased peripheral artery stiffness, reflection points move closer to the heart which results in the summated wave arriving earlier, in systole, rather than diastole (69). These disturbed wave reflections contribute to increased SBP and PP and inhibit coronary perfusion (70). Further, there is evidence to suggest that this increased pressure can be transmitted to the microcirculation, causing damage (71,72). As such faPWV may provide useful insight into the interaction between the central and peripheral vasculature as well as an indication of changes in endothelial function within the lower limb.

Counterintuitively, there was a significant reduction in AIx in response to the uninterrupted sitting bout which looks to be driven by a reduction in Pb (**Table 2**). This reduction in AIx, suggesting a decrease in arterial stiffness, is seemingly incongruent with the reported PWV results from the current study and others (51,59,62,63,73). The reason for this incongruence is unclear, however, it is possible that unmeasured changes in local or central haemodynamics may have influenced the derivation of AIx from the brachial waveform (74). Indeed, these results also conflict with previous prolonged sitting studies which have assessed AIx using more direct methods (59). Further work is needed to fully explore the effect of prolonged uninterrupted sitting on AIx as an index of arterial stiffness.

It is important to note that the changes in both central and peripheral arterial stiffness observed in this study and others are small, and it would be speculative to infer the long-term effects of these acute changes on future cardiovascular health or disease risk. However, the current working model linking

sedentary behaviour to future cardiovascular disease risk posits that these acute bouts of dysfunction temporarily increase the burden on the cardiovascular system as a whole (30,31). With increased stiffness, the central arteries are less able to buffer the pulsatile force of the heart and the capacity of the peripheral arteries to serve as conduits is impaired (70,75). In the context of one sitting bout, these changes are minute, however, given that sedentary behaviours such as sitting comprise a majority of many people's waking hours, it is likely that people are exposed to this negative phenotype repeatedly throughout each day and throughout their lives which may ultimately present as increased cardiovascular disease risk in the future.

Comparison to Literature: Associations of Cardiorespiratory Fitness and Habitual Physical Activity

Previous research investigating the effect of CRF on sitting-induced changes in cardiovascular health and function has principally focused on popliteal FMD (17,18), and microvascular function using the passive leg technique (16). Owing to the associations between endothelial dysfunction and increased arterial stiffness (22), faPWV reported in this study is a useful, and reliable measure for comparison (50). In agreement with Garten et al., (16) the present study found no association between CRF and sitting-induced dysfunction. However, it should be noted that research in this area has reached equivocal conclusions. Morishima et al., (17) concluded that CRF conferred a protective effect against sitting-induced reductions in popliteal artery FMD, whereas Liu et al., (18) concluded that increased CRF was associated with increased sitting-induced popliteal artery dysfunction. The reason for these divergent findings is unclear but one possibility could be the product of the proclivity for error associated with FMD (76–78). Additionally, FMD assessments are arguably a snapshot, only indicative of the local area being analysed. By contrast, faPWV summates the properties of a greater portion of the lower limb vasculature. Alternatively, a lack of standardisation in how vascular assessments have been performed may have contributed to the equivocal findings in this research line.

As discussed by our group and others, there is currently a lack of standardisation within sitting research as it pertains to the posture that vascular function assessments are performed, with some researchers opting for seated assessments and others opting for supine (79–81). Indeed, in the current paper and Morishima et al., (17), all vascular assessments were performed with participants in a supine posture following a post-sitting rest period, whereas in Garten et al., (16) and Liu et al., (18) conducted measurements with participants seated. Differences in autonomic nervous system (ANS) activity as a product of posture differences may contribute to the conflicting findings. It is well established that a seated posture increased ANS activity relative to a supine posture (82,83) and that muscle sympathetic nervous activity (MSNA) increases over time during an orthostatic stress such as sitting (84). It is known that MSNA impacts vascular resistance and conductance (85) and that CRF appears to influence that relationship (86,87). As such, it is conceivable that in seeking to address the question of fitness levels and their effect on vascular responses to prolonged uninterrupted sitting, measures taken in different

postures may not be readily compared. This further highlights the need for consistent standardisation within prolonged sitting research.

Implications

The long-term benefits of CRF and HPA are thought to be the product of reduced systemic inflammation and exposure to regular bouts of increased blood flow, and therefore shear stress, facilitating improved endothelial function (88–92). From the results of this study, it does not appear that the benefits associated with increased CRF or HPA carry over to acute bouts of sedentary behaviour. However, previous research has shown that physical activity immediately before bouts of prolonged sitting confers a protective effect (93,94). Therefore, the results of this study should not be interpreted as suggesting that CRF and HPA are not important targets for individuals. Indeed, previous research has demonstrated the negative effects of acutely reduced physical activity on vascular function (19) as well as the beneficial effects of acute increases in physical activity (95). Instead, these results indicate that CRF and HPA levels of participants may not be a major consideration when recruiting for larger studies in the future, however, this assertion should be investigated in a larger sample before being implemented.

Conclusions

The results of this study suggest that 2-hours uninterrupted sitting significantly impairs lower-limb PWV, however, neither CRF nor HPA, as assessed via daily step counts, LPA, or MVPA influenced such increases.

Data Availability

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

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Disclosures

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

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

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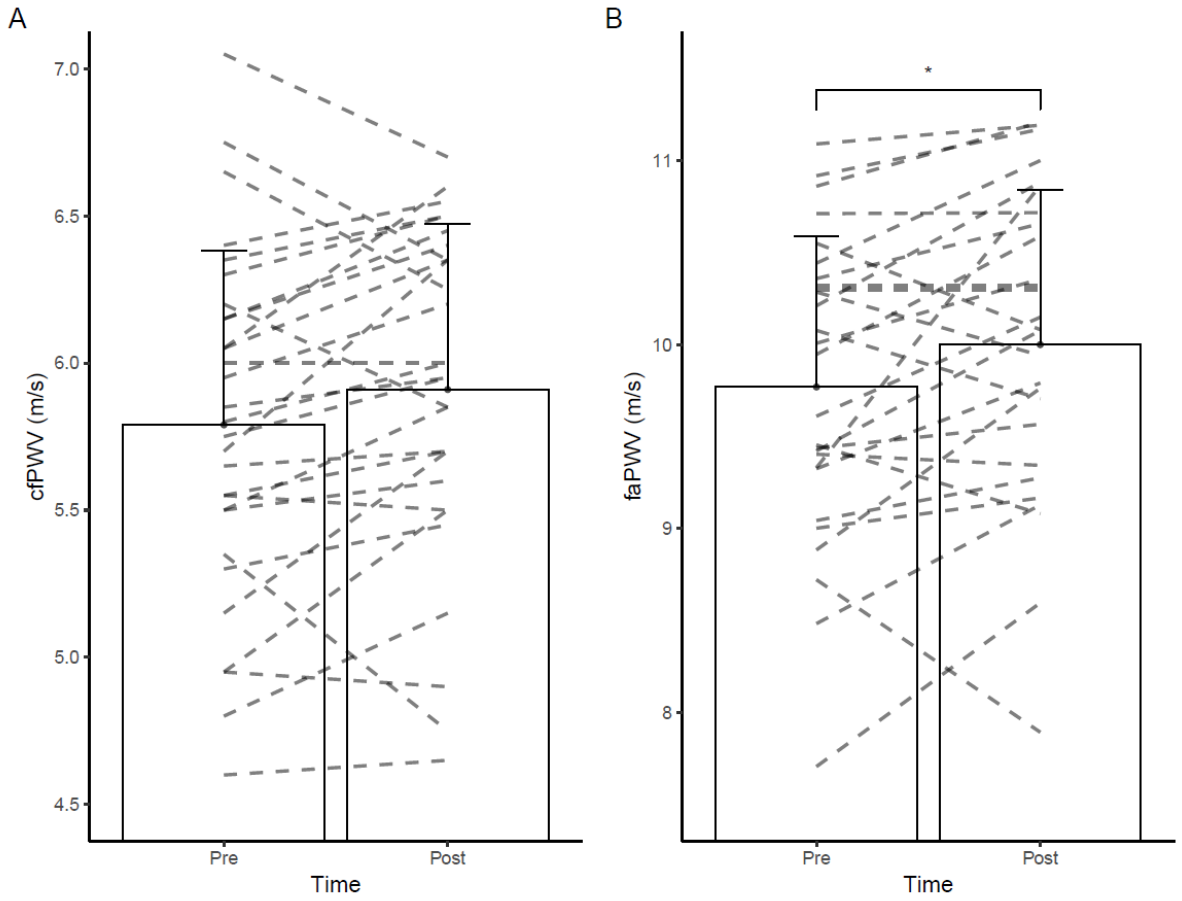
Figure Legends

Figure 1. A) Changes in carotid-femoral pulse wave velocity in response to 2 h of uninterrupted sitting. B) Changes in femoral-ankle pulse wave velocity in response to 2 h of uninterrupted siting. Group data are presented as mean and SD. Individual participant data are presented as individual dashed lines.


A
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cfPWV (m/s)
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faPWV (m/s)
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Table 1 Characteristics of included participants

	Mean (SD)
Participant Characteristics	
Age (y)	32 (7)
Stature (m)	1.75 (0.1)
Mass (kg)	79.1 (11.5)
Body Mass Index ($kg.m^2$)	26 (3)
Cardiorespiratory fitness	
Absolute $\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	4.2 (0.8)
Relative $\dot{V}O_{2max}$ ($mL \cdot kg \cdot min^{-1}$)	52.6 (8.6)
Peak respiratory exchange ratio	1.15 (0.06)
Habitual physical activity and sedentary behaviours	
Daily step count ($steps \cdot day^{-1}$)	10,332 (2,927)
Light-intensity physical activity (mins/day)	54 (19)
Moderate-to-vigorous physical activity (mins/day)	36 (21)
Sedentary time (mins/day)	645 (125)
Sitting time (mins/day)	496 (89)

Table 2 Supine pulse wave analysis, pulse wave velocity, and near-infrared spectroscopy responses to 120 mins prolonged uninterrupted sitting

Measure	Pre	Post	MD (SE)	*p*	*d*
cfPWV ($m \cdot s^{-1}$)	5.8 (0.6)	5.9 (0.6)	0.12 (0.06)	0.078	0.38
faPWV ($m \cdot s^{-1}$)	9.8 (0.8)	10.0 (0.8)	0.26 (0.10)	**0.037***	0.51
SBP (mmHg)	113 (11)	116 (9)	2.50 (1.33)	0.099	0.35
DBP (mmHg)	66 (7)	69 (7)	2.83 (1.08)	**0.037***	0.49
MAP (mmHg)	79 (7)	80 (7)	1.78 (0.95)	0.096	0.36
cSBP (mmHg)	101 (9)	101 (8)	0.83 (1.12)	0.510	0.15
cDBP (mmHg)	67 (7)	69 (7)	2.45 (0.99)	**0.046***	0.47
cMAP (mmHg)	77 (7)	81 (7)	1.95 (0.94)	0.078	0.39
cPP (mmHg)	33 (6)	32 (5)	-1.22 (0.59)	0.060	0.43
AIx (%)	9 (12)	2 (12)	-7.09 (1.03)	**0.011***	1.31
AIx@75 (%)	-0.6 (12)	-8 (13)	-7.47 (1.36)	**0.011***	1.02
Pf (mmHg)	24 (4)	24 (4)	0.29 (0.62)	0.729	0.09
Pb (mmHg)	12 (2)	11 (2)	-1.09 (0.26)	**0.013***	0.77
HR (BPM)	54 (7)	54 (9)	0.05 (0.73)	0.944	0.01
HHb (µmol)	16 (7)	18 (7)	1.92 (0.57)	**0.011***	0.65
tHB (µmol)	59 (19)	58 (18)	-0.48 (1.19)	0.739	0.07

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; cMAP, central mean arterial pressure; cPP, central pulse pressure; AIx, augmentation index; HR, heart rate; BPM, beats per minute; HHb, deoxygenated haemoglobin; tHB, total haemoglobin. * denotes statistical significance

Table 3 Results of Partial Pearson correlations between cardiorespiratory fitness and habitual physical activity versus sitting-induced changes in carotid-femoral and femoral-ankle pulse wave velocity

Measure	Cardiorespiratory Fitness		Habitual Physical Activity					
			Daily Step Count		LPA		MVPA	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
cfPWV	0.105	0.981	-0.228	0.675	-0.084	0.981	-0.005	0.981
faPWV	0.009	0.981	-0.377	0.252	-0.507	0.088	0.059	0.981

Abbreviations: cfPWV, carotid-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous intensity physical activity. Note: n = 29 for CRF comparisons and n = 28 for HPA comparisons.