Word count main text: 4779

Word count main abstract: 450

Number of references: 71

Figures: 4

Tables: 4

Electronic Supplementary Material: 1

Title: The effects of acute exposure to prolonged sitting, with and without interruption, on vascular function among adults: A meta-analysis

Running head: Acute sitting exposure and FMD

Type: Systematic Review

Authors: Craig PATERSON[1*], Simon FRYER[1], Gabriel ZIEFF[2], Keeron STONE[1], Daniel P CREDEUR[3], Bethany BARONE GIBBS[4], Jaume PADILLA[5,6], John K. Parker[1], Lee STONER[2]

[1] School of Sport and Exercise, University of Gloucestershire, Gloucester, UK.

[2] Department of Exercise and Sports Science, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

[3] School of Kinesiology and Nutrition, University of Southern Mississippi, Hattiesburg, MS, USA.

[4] Department of Health and Physical Activity, University of Pittsburgh, Pittsburgh, PA, USA.

[5] Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, USA.

[6] Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, USA.

[*] Corresponding Author: E: craigpaterson@connect.glos.ac.uk

ORCID IDs

Craig Paterson - 0000-0003-3125-9712

Simon Fryer - 0000-0003-0376-0104

Gabriel Zieff - 0000-0002-4043-2911

Keeron Stone - 0000-0001-6572-7874

Daniel P. Credeur – 0000-0002-5084-2666

Bethany Barone Gibbs - 0000-0002-0732-6148

Jaume Padilla - 0000-0002-7944-4936

John K. Parker - 0000-0003-4912-1535

Lee Stoner - 0000-0002-0682-2270

Conflicts of interest: None

Source of funding: None

Abstract

Background: Exposure to acute prolonged sitting can result in vascular dysfunction, particularly within the legs. This vascular dysfunction, assessed using flow-mediated dilation (FMD), is likely the consequence of decreased blood flow-induced shear stress. With mixed success, several sitting interruption strategies have been trialled to preserve vascular function.

Objectives: The objectives of this study were to (1) assess the effects of acute prolonged sitting exposure on vascular function in the upper- and lower-limb arteries, and (2) evaluate the effectiveness of sitting interruption strategies in preserving vascular function. Sub-group analyses were conducted to determine whether artery location or interruption modality explain heterogeneity.

Data Sources: Electronic databases (PubMed, Web of Science, SPORTDiscus, and Google Scholar) were searched from inception to January 2020. Reference lists of eligible studies and relevant reviews were also checked.

Study Selection: Inclusion criteria for objective (1) were: (i) FMD% was assessed pre- and post-sitting; (ii) studies were either randomised-controlled, randomised-crossover, or quasi-experimental trials; (iii) the sitting period was ≥1 hour; (iv) participants were healthy non-smoking adults (≥ 18 years), and free of vascular-acting medication and disease at the time of testing. Additional inclusion criteria for objective (2) were: (i) the interruption strategy must have been during the sitting period, (ii) there was a control (uninterrupted sitting) group/arm, and (iii) the interruption strategy must have involved the participants actively moving their lower- or upper-limbs.

Appraisal and synthesis methods: 1776 articles were identified, of which 17 (22 trials, *n*=269) met inclusion criteria for objective (1). Of those, 17 articles (9 trials, *n*=127) met the inclusion criteria for objective (2). Weighted mean differences (WMD), 95% confidence intervals (95% CI), and standardised mean difference (SMD) were calculated for all trials using random-effects meta-analysis modelling. SMD was used to determine the magnitude of effect, where <0.2, 0.2, 0.5, and 0.8 was defined as trivial, small, moderate, and large respectively.

Results: (1) Random-effects modelling showed uninterrupted bouts of prolonged sitting resulted in a significant decrease in FMD% (WMD=-2.12%, 95% CI:-2.66 to -1.59, SMD=0.84). Subgroup analysis revealed reductions in lower- but not upper-limb FMD%. (2) Random-effects modelling showed that interrupting bouts of sitting resulted in a significantly higher FMD% compared to uninterrupted sitting (WMD=1.91%, 95% CI:0.40, 3.42,

SMD=0.57). Subgroup analyses failed to identify an optimum interruption strategy but revealed moderate non-significant effects for aerobic interventions (WMD=2.17%, 95% CI:-0.34 to 4.67, SMD=0.69) and simple resistance activities (WMD=2.40%, 95% CI:-0.08 to 4.88, SMD=0.55) and a trivial effect for standing interruptions (WMD=0.24%, 95% CI:-0.90 to 1.38, SMD=0.16).

Conclusions: Exposure to acute prolonged sitting leads to significant vascular dysfunction in arteries of the lower, but not upper limbs. The limited available data indicates that vascular dysfunction can be prevented by regularly interrupting sitting, particularly with aerobic or simple resistance activities.

Conflicts of interest: None
Source of funding: None

Key Points

- Contemporary evidence suggests that bouts of prolonged sitting can result in vascular dysfunction, a precursor to cardiovascular disease. Prior to this analysis, the average magnitude of vascular dysfunction following prolonged sitting and how it may differ across arteries was unclear.

- This meta-analysis shows that bouts of prolonged sitting create significant dysfunction in lower- but not upper-limb arteries and that this dysfunction can be prevented by interrupting prolonged sitting with simple resistance or aerobic interruption strategies.

- Further work is needed to identify the optimal dose and frequency of interruptions.

1. Introduction

Chronic exposure to sedentary behaviour, characterised by low energy expenditure (≤ 1.5 metabolic equivalents in a seated, reclined, or lying posture [1]) has been associated with increased cardiovascular disease (CVD) incidence and mortality [2,3]. The mechanisms linking repeated exposure to prolonged sedentary behaviour, particularly sitting, and CVD risk are not fully understood. However, there is sufficient evidence to indicate that acute prolonged sitting results in transient vascular dysfunction [4–20]. Recent studies report that flow-mediated dilation (FMD), the gold-standard non-invasive assessment of endothelial health and a marker of vascular function, can be reduced transiently by up to an absolute 5% (i.e., 22% to 17%) following prolonged sitting [8]. Whether transient reductions in vascular function as a result of sitting have prognostic implications remains unclear; however, it should be noted that transient endothelial dysfunction induced by other insults (e.g., mental stress) has recently been associated with future cardiovascular events in patients with stable coronary artery disease [21]. It is also established that chronic reductions in FMD are associated with up to a 13% increase in the risk of future cardiovascular events in individuals with and without established CVD [22–25].

One potential mechanism linking acute prolonged sitting to decreased vascular function is decreased blood flow-induced shear stress [16,26]. A number of studies have explored strategies to interrupt sitting and stimulate increases in blood flow to the inactive limbs [5–7,9,10,17]. These interruption strategies have included leg fidgeting [10], body weight resistance exercise [7], desk-based cycling [9], walking [5,17], standing [9], and callisthenics [6]. Whilst some studies individually have shown preservation of FMD [5–7,10,17], the optimum strategy is unclear. In addition, the FMD response has been assessed in a range of different arteries, further complicating the interpretation of findings. Gaining an understanding of the degree to which upper and lower limb arteries are affected, and which sitting interruption strategies are most efficacious is important for guiding evidence-based practices for decreasing CVD risk in an increasingly sedentary population [27–35].

1.1 Objectives

The current meta-analysis aimed to consolidate the existing literature to determine the effects of sitting, both uninterrupted and interrupted, on vascular function. The objectives were two-fold: (1) to conduct a meta-analysis to determine the effect of uninterrupted sitting on vascular function, with subgroup analysis to identify whether the artery assessed helped to explain heterogeneity in the analysis; and (2) to conduct a separate meta-analysis to determine the effect of interrupted sitting on vascular function, with additional subgroup analysis of artery, and interruption strategy to explain heterogeneity.

2 Methods

This meta-analysis was reported in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [36].

2.1 Data Sources and Searches

Electronic databases (PubMed, Web of Science, SPORTDiscus, and Google Scholar) were searched by two authors (CP and GZ) utilising the keywords: (sitting OR prolonged sitting OR sedentary OR sedentary behaviour) AND (vascular function OR endothelial function OR endothelium function OR endothelial dysfunction OR endothelium dysfunction OR flow mediated dilation OR flow mediated vasodilation OR flow dependent dilation OR flow dependent vasodilation OR vascular reactivity OR FMD). The reference lists of all identified trials and relevant reviews were also examined. The search was limited to English Language studies published between inception and January 2020.

2.2 Article Selection

For the purpose of this meta-analysis, the terms 'article' and 'study' are used synonymously; 'trial' is the unit included in the meta-analysis. A given article may have resulted in more than one eligible trial if the article included more than one intervention group or FMD assessment site. Article titles and abstracts were screened for relevance, and duplicate studies were removed before obtaining the full text of potentially eligible articles to review for inclusion. The following criteria were used to select trials for inclusion in the review: (i) FMD was assessed pre- and post-sitting; (ii) studies were either randomised controlled, randomised crossover, or quasi-experimental pre- versus post-test trials; (iii) the prolonged sitting period was at least one hour; (iv) participants were non-smoking adults ($\geq$ 18 years of age), not taking any vascular acting medication, and were considered healthy, having no major acute or chronic illness. To address the second objective of this review regarding sitting interruption strategies, further additional criteria were used; (i) if a strategy was employed to disrupt the effects of sitting, the strategy must have been during the sitting period; (ii) there must have been a control (uninterrupted sitting) group or condition, and (iii) the interruption strategy must have involved the participants actively moving either the lower or upper limbs. If a study employed an intervention prior to or after the sitting period, only data from the control (uninterrupted sitting) trial was included in the analysis. Two researchers completed the study selection independently (CP and GZ).

2.3 Data Extraction and Quality Assessment

Data extracted for each eligible trial included bibliographic information (author, publication year), collected measures, sample characteristics (age, sex, body mass index, etc.), details of any interventions, arterial site, and uncorrected relative FMD values. Uncorrected FMD values were collected, as opposed to allometrically scaled or normalized to the shear rate stimulus, as there is a lack of consensus on the correction process and different

approaches have been used. If these data were not included in the article, the investigators contacted the authors for further information. Data extraction was completed independently by two researchers (CP and GZ). Study quality was assessed using the Cochrane Risk of Bias Tool [37] and a modified Heyland Methodological Quality Score (HMQS) [38,39] with a maximum score of 10 (Electronic Supplementary Material Appendix S1). Two additional levels were added to the standard HMQS, relating to the described FMD assessment. These extra levels addressed whether the measure was performed in the recommended and validated supine posture, and whether appropriate guidelines were followed [40–42]. Due to the technical aspects of assessing FMD, adherence to published guidelines are imperative to ensure reliable and reproducible results [42,43]. With respect to existing HMQS criteria, as blinding of participants is not feasible, blinding of the operator assessing FMD was considered a quality criterion for the HMQS as opposed to participant blinding. The HMQS criteria "extent of follow up", "cointerventions", and "outcomes" were removed from the current analysis as they are designed for longitudinal studies. Quality assessment was completed independently by two researchers (CP and SF), with consultation from a third researcher (LS) in the case of discrepancies.

2.4 Data Synthesis

For the outcome of interest, the pre- and post-intervention values (mean and standard deviation) as well as mean differences and associated standard deviations were entered into a spreadsheet. When data were not published, a request of the missing values was made to the corresponding author and following non-response the values were estimated based on methods from the Cochrane Handbook for Systematic Reviews of Interventions [37]. For studies reporting multiple time points during the bout of sitting, only the pre-trial and final time point values were used in analysis. Aggregation and calculation of final results was conducted by two authors (CP and SF).

2.5 Data Analysis

All extracted data were entered into software specifically designed for meta-analyses (MetaXL, http://www.epigear.com/index_files/metaxl.html). Outcome measures were calculated as weighted mean differences (WMDs) as well as the standardised mean difference (SMD). The SMD was used to determine the magnitude of the effect, where <0.2, 0.2, 0.5, and 0.8 was defined as trivial, small, moderate, and large respectively [42]. Random-effects modelling, with the DerSimonian-Laird method, was used for both analyses as it allows for heterogeneity in experimental procedures and accounts for both within- and between-trial variance [44]. The statistical heterogeneity across trials included in the meta-analysis was assessed using the I^2 statistic, where <25%, 25%, and 75% represent low, moderate, and considerable heterogeneity, respectively [45]. Sensitivity analyses were performed by excluding one trial at a time to test the robustness of the pooled results. The Luis Furuya-Kanamori (LFK) index was used as it is a means of identifying and quantifying asymmetry and potential small

study bias, where <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry [46]. Additionally, publication bias was evaluated by visual inspection of the Begg's funnel plot when (i) at least 10 trials were included in the meta-analysis, and (ii) there was substantial variation in sample size for the included trials [37]. Two authors (LS and CP) conducted the data analysis.

3 Results

3.1 Literature Search and Trial Selection

The literature search strategy is outlined in Figure 1. Initial database searches identified a total of 1797 potentially eligible articles with a further 5 identified through manual searches. Following screening of titles and abstracts, 1769 articles were excluded because they did not meet inclusion criteria. The remaining 33 papers underwent full text screening and 16 further studies were excluded. The final analysis included 17 studies (22 trials) for objective (1), of which 6 (9 trials) were included in a separate analysis of interruptions to prolonged sitting for objective (2).

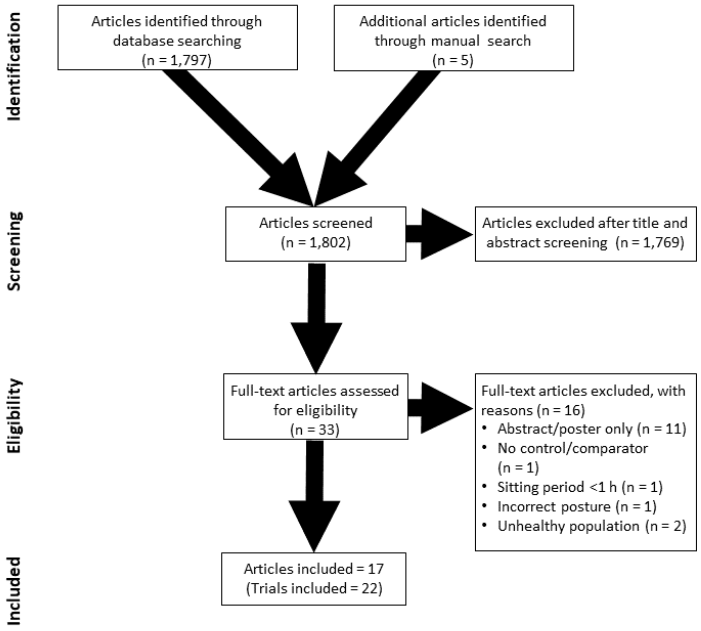

Figure 1 Flow chart presenting the inclusion and exclusion criteria

3.2 Characteristics of Included Studies

The trial characteristics are summarised in Table 1. The number of participants in each trial ranged from 8 [14] – 20 [8]. Of the 22 trials, 12 included only male participants [4,12–20], and 2 included only females [13,20], with

8 trials included both sexes [5–8,10,11]. Bouts of prolonged sitting ranged from 1.5 [6] to 6 hours [15], with a modal sitting duration of 3 [4,10–14,16–20]. Assessments of FMD were carried out predominantly in the lower limb, with only 4 of the 18 trials assessing brachial artery (BA) FMD [6,7,15,18]. Of the trials that assessed FMD in the lower limb, 6 assessed the superficial femoral artery (SFA) [4,5,7,17–19], and 11 assessed the popliteal artery (PA) [9–15,20], with 1 assessing the posterior tibial artery (PTA) [8]. Of the 18 trials, 9 included strategies to interrupt the bout of prolonged sitting [5–7,9,10,17]. These interruptions were categorised as aerobic [5,9,10,17], simple resistance activities [6,7], or standing [9].

3.3 Methodological Quality Assessment

The methodological assessment of included trials is summarised in Table 1. The quality of studies ranged from 3 to 8 out of a possible maximum of 10, with the median quality score being 7. All trials assessed and reported all collected data and reported any dropouts or unusable data. For blinding, 10 trials reported that offline analysis of FMD videos was performed by a blinded technician/researcher [4,7,13,14,17–19]. Ten trials reported the published FMD guidelines that they adhered to [5,6,8,11,13,17–19], and 8 trials performed FMD with participants in the suggested supine position [5,6,8–12,16].

3.4 Synthesis of the Results

3.4.1 Effects of Prolonged Sitting on Flow-Mediated Dilation

Prolonged sitting resulted in a large and significant decrease in FMD% (WMD = -2.12%, 95% Confidence Intervals (CI): -2.66 to -1.59, $p < 0.001$, SMD = -0.84) (Figure 2). Sensitivity analysis indicated that none of the trials unduly influenced the observed outcome. Visual inspection of the funnel plot did not reveal substantial asymmetry (Figure 3), although the LFK index of 1.36 did indicate minor asymmetry. The heterogeneity was moderate ($I^2 = 43\%$, $p < 0.019$), which may be partially explained by FMD being assessed on different arteries, including upper- and lower-limb arteries. Subgroup analysis revealed moderate and large significant decreases in SFA and PA FMD%, respectively (SFA, WMD = -1.75%, SMD = -0.59; PA, WMD = -2.51%, SMD = -1.41). There was a non-significant small decrease in PTA FMD% (WMD = -5.00%, SMD = -0.37), and a non-significant trivial increase in BA FMD% (WMD = 0.03%, SMD = -0.02) (Table 2).

Table 1 Characteristics of the included trials

Ref	Quality	Sample [n (F); mean age, years (SD)]	FMD assessment site	Sitting duration (h)	Interruption strategy
Ballard et al. [4]	6	11 (0); 21.2 (1.9)	SFA	3	N/A
Carter et al. [5]	7	15 (5); 35.8 (10.2)	SFA	4	Aerobic
Carter et al. [6]	7	10 (4); 27.3 (8.1)	BA	1.5	SRA
Climie et al. [7]a	8	19 (8); 57 (12)	SFA	5	SRA
Climie et al. [7]b	8	19 (8); 57 (12)	BA	5	SRA
Credeur et al. [8]	6	20 (7); 26 (7)	PTA	3	N/A
Kruse et al. [9]	7	13 (3); 38 (3)	PA	4	Aerobic and Standing
Morishima et al. [10]	7	11 (4); 26 (1)	PA	3	Aerobic
Morishima et al. [11]	8	15 (5); 26.7 (0.5)	PA	3	N/A
Morishima et al. [12]	7	9 (0); 21.2 (2)	PA	3	N/A
O'Brien et al. [13]a	6	10 (0); 24 (2)	PA	3	N/A
O'Brien et al. [13]b	6	10 (10); 23 (2)	PA	3	N/A
Padilla et al. [14]	7	8 (0); 24 (1.7)	PA	3	N/A
Restaino et al. [15]a	4	11 (0); 27 (1)	PA	6	N/A
Restaino et al. [15]b	4	11 (0); 27 (1)	BA	6	N/A
Restaino et al. [16]	7	10 (0); 26 (1)	PA	3	N/A
Thosar et al. [17]	7	12 (0); 24.2 (4.2)	SFA	3	Aerobic
Thosar et al. [18]a	5	12 (0); 24.2 (4)	BA	3	N/A
Thosar et al. [18]b	5	12 (0); 24.2 (4)	SFA	3	N/A
Thosar et al. [19]	7	11 (0); 24.2 (4.4)	SFA	3	N/A
Vranish et al. [20]a	3	12 (12); 20 (0)	PA	3	N/A
Vranish et al. [20]b	3	8 (0); 22 (1)	PA	3	N/A

Abbreviations: F, females; SD, standard deviation; FMD, flow-mediated dilation; SFA, superficial femoral artery; BA, brachial artery; PTA, posterior tibial artery; PA, popliteal artery; SRA, simple resistance activities; N/A, not applicable. Quality was assessed using a modified Heyland Methodological Quality Score, with a maximum score of 10. Labels a and b denotes different trials from the same study.

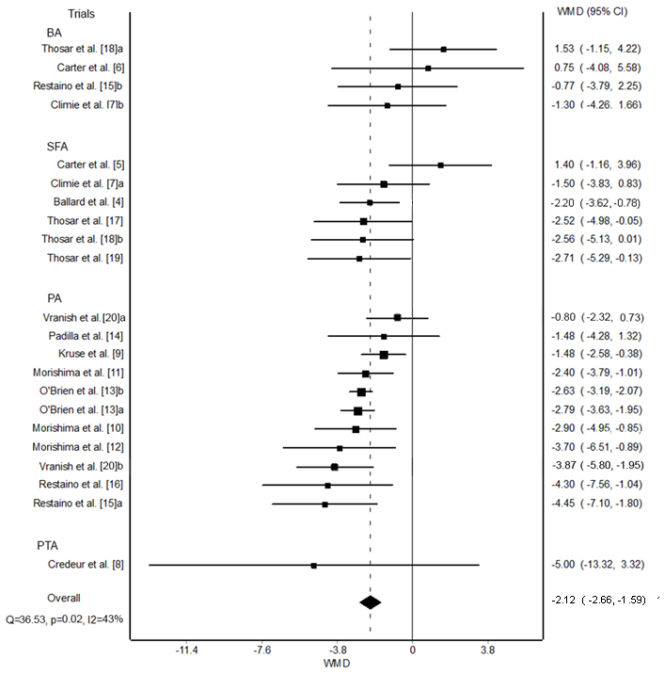

Figure 2 The effect of prolonged uninterrupted sitting on vascular function meta-analysis using a random-effects model grouped by artery.

Abbreviations: WMD, weighted mean difference; CI, confidence intervals. Labels a and b denotes different trials from the same study. BA, brachial artery; SFA, superficial femoral artery; PA, popliteal artery; PTA, posterior tibial artery. Labels a and b denotes different trials from the same study.

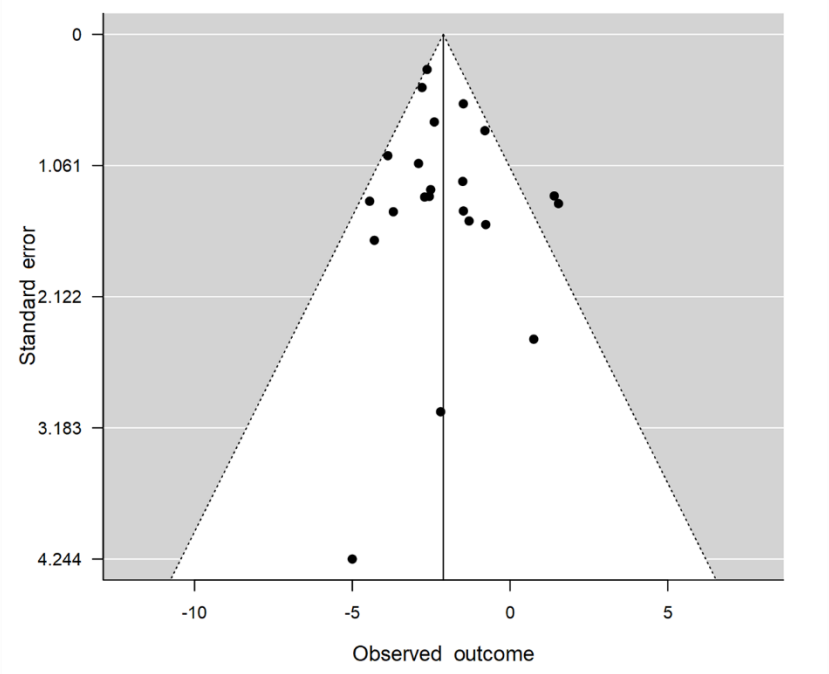

Figure 3 Funnel plot for uninterrupted sitting meta-analysis.

3.4.2 Effects of Sitting Interruption on the Flow-Mediated Dilation Response to Prolonged Sitting

Across sitting interruption strategies there was a moderate, significantly greater FMD% for the experimental (interrupted) conditions compared to the control (uninterrupted) (WMD = 1.91%, 95% CI: 0.40 to 3.42, $p = 0.01$, SMD = 0.57) (Figure 4). Sensitivity analysis indicated that removal of any one of 3 trials [7,10,17] resulted in a reduced overall effect but did not result in a loss of statistical significance. An LFK index of 3.36 indicated major asymmetry. The heterogeneity for this analysis was considerable ($I^2 = 79\%$, $p < 0.001$) and may be explained by the low number of trials, testing FMD on different arteries, and the use of varying interruption strategies. With respect to different arteries, subgroup analysis revealed non-significant effects on BA, SFA, and PA FMD% (Table 3). This analysis also revealed considerable heterogeneity in the SFA and PA subgroups ($I^2 = 77\%$ and 90% respectively). With regards to sitting interruption strategies, simple resistance activities and aerobic interruption strategies resulted in non-significant moderately greater FMD% compared to control conditions (Table 3). This subgroup analysis also revealed considerable heterogeneity for the aerobic subgroup ($I^2 = 86\%$) and moderate heterogeneity for the simple resistance activities subgroup ($I^2 = 47\%$). Finally, only one trial included standing as an interruption strategy [9], reporting a non-significant difference in FMD% between conditions (WMD = 0.24, 95% CI: -0.90 to 1.38) (Table 3)

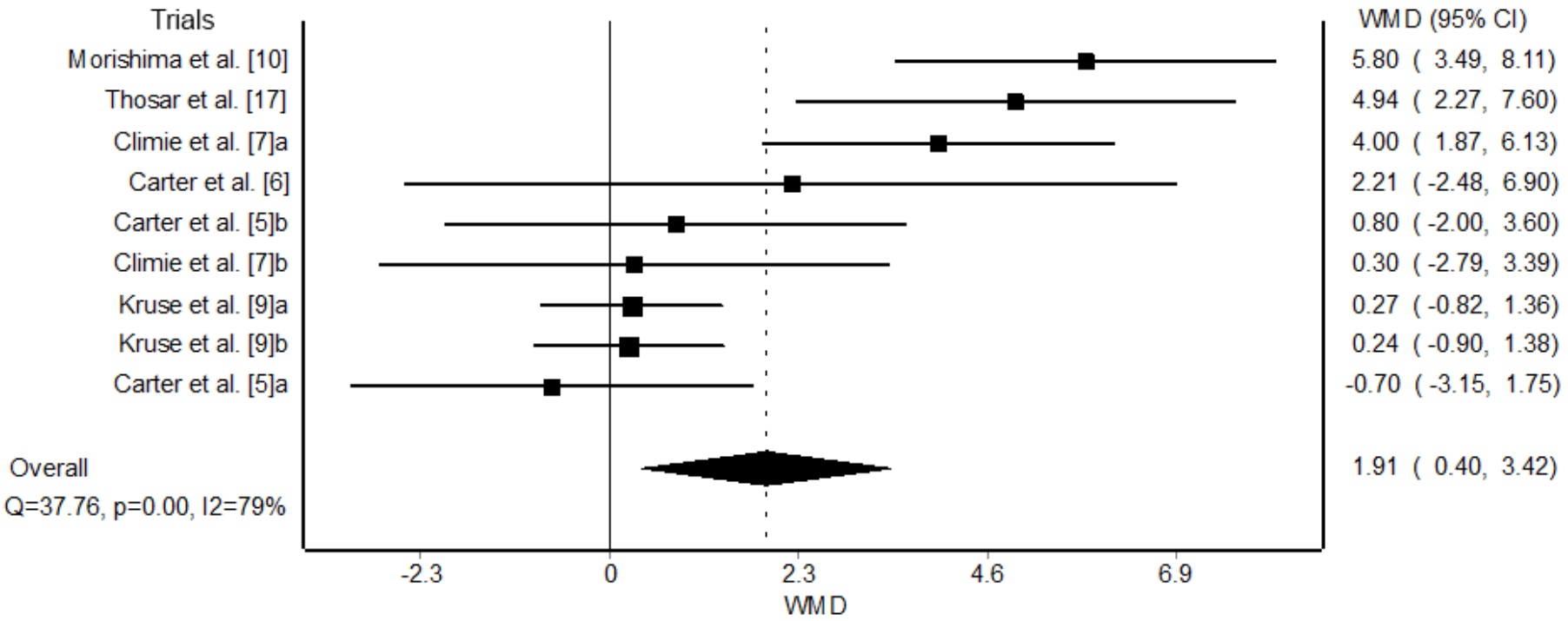

Figure 4 The effect of interrupted prolonged sitting on vascular function meta-analysis using a random-effects model

Abbreviations: WMD, weighted mean difference; CI, confidence intervals. Labels a and b denotes different trials from the same study.

Table 2 Uninterrupted sitting with subgroup analysis by artery using a random-effects meta-analysis model

	Pooled Effect					Heterogeneity			Asymmetry			
	WMD	LCI	UCI	P Value	SMD	Q	P Value	I^2	LFK	Quality	Trials	Sample
All	-2.12	-2.66	-1.59	<0.001	-0.84	36.5	0.02	43	1.36	6	22	269
Artery												
SFA	-1.75	-2.88	-0.63		-0.59	7.50	0.19	33		7	6	80
BA	0.03	-1.54	1.60		-0.02	2.34	0.51	0		6	4	52
PTA	-5.00	-13.32	-3.32		-0.37	N/A	N/A	N/A		6	1	20
PA	-2.51	-3.06	-1.97		-1.41	15.3	0.12	35		6	11	117

Abbreviations: WMD, weighted mean difference; LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SFA, superficial femoral artery; BA, brachial artery; PTA, posterior tibial artery; PA, popliteal artery; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry. I2: 25%, 50%, and 75% represent low, moderate, and high heterogeneity respectively.

Table 3 Interrupted sitting with subgroup analysis by artery and interruption strategy using a random-effects meta-analysis model

	Pooled Effect					Heterogeneity			Asymmetry			
	WMD	LCI	UCI	P Value	SMD	Q	P Value	I^2	LFK	Quality	Trials	Sample
All	1.91	0.40	3.42	0.01	0.57	37.8	<0.001	79	-3.36	7	9	127
Artery												
SFA	2.28	-0.32	4.88		0.65	13.1	<0.001	77		8	4	31
BA	0.88	-1.70	3.46		0.18	0.44	0.51	0		8	2	29
PA	1.86	-0.68	4.40		0.76	19.8	<0.001	90		7	3	37
Interruption Strategy												
SRA	2.40	-0.08	4.88		0.55	3.78	0.15	47		8	3	48
Aerobic	2.17	-0.34	4.67		0.69	27.8	<0.001	86		7	5	66
Standing	0.24	-0.90	1.38		0.16	N/A	N/A	N/A		7	1	13

Abbreviations: WMD, weighted mean difference; LCI, lower confidence interval; UCI, upper confidence interval; SMD, standardised mean difference; LFK, Luis Furuya-Kanamori Index; SFA, superficial femoral artery; BA, brachial artery; PA, popliteal artery; SRA, simple resistance activities; N/A, not applicable. SMD: Trivial, small, moderate and large effect sizes are defined as <0.2, 0.2, 0.5, and 0.8 respectively. LFK: <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry. I2: 25%, 50%, and 75% represent low, moderate, and high heterogeneity respectively.

4 Discussion

The aim of this meta-analysis was to synthesise existing data with respect to the effects of prolonged sitting (>1 hr), with and without interruption, on vascular function in adults. The main findings were that: (1) prolonged uninterrupted sitting resulted in a significant decrease (detrimental) in FMD% (WMD = -2.12%, 95% CI: -2.66 to -1.59, SMD = 0.84), with these effects occurring in the lower limbs (SFA, PA, and PTA), but not in the upper limb (BA); and (2) regular interruptions to sitting appear to confer a protective effect against vascular dysfunction, however, the optimum interruption strategy cannot yet be identified at this time due to the limited number of trials.

4.1 Limitations

Whilst this meta-analysis has produced meaningful information, several potential limitations should be acknowledged when interpreting the results of this analysis. Firstly, there was a limited number of eligible trials and the sample sizes were small (range = 8 – 20, median = 12). Additionally, only 4 trials reported sample size calculation for the FMD outcome [4,16,17,19]. However, this is the first meta-analysis looking at the effects of prolonged sitting with and without interruption and some important methodological insights for future studies are noted. Second, our analysis considered change in FMD%. FMD% is a ratio calculated by dividing the maximum change in artery diameter in response to reactive hyperaemia by the resting artery diameter. This approach has been criticised as the change in diameter is inversely proportional to baseline diameter and thus a ratio is statistically unsuitable [47]. Allometric scaling has been offered as a means of controlling for the influence of baseline diameter , however this approach may not be able to adequately correct FMD% in different vascular beds or at an individual level, i.e. it can only be applied to group means [42,48,49]. An alternate approach has been to correct FMD% by using shear rate as a covariate [50,51], though this approach has also been suggested to have limitations [52]. These points, taken together, indicate that there is a current lack of consensus about the best statistical strategy for controlling for factors that influence FMD. Consequently, current FMD guidelines still suggest reporting FMD% irrespective of any further analysis [40,41]. Subsequently, these data are readily available within the literature and, whilst certain limitations of the metric are acknowledged, FMD% served as an appropriate metric for this analysis. Thirdly, this study has highlighted a sex-specific void in the research. Specifically, 75% of the overall sample were male, and of the 10 trials which included females, 6 studied females in the follicular phase of the menstrual cycle [5,6,8,9,13,20], 2 did not control for menstrual cycle [10,11], and 2 studied menopausal women [7]. Given the potential influence of the menstrual cycle on FMD% [53–57], and the small number of females sampled, generalising the findings of this meta-analysis to females is difficult. Lastly, this analysis only considered the difference between baseline and final FMD%, so any inferences regarding the time course of vascular dysfunction during uninterrupted sitting cannot be made. This practice was based on ~70%

of trials only implementing pre- and post-sitting FMD assessments [5,6,8–16,20] and the indication by current expert guidelines that participants should be supine for FMD assessments [40,42]. Consequently, any posture transitions to facilitate repeated assessments would not constitute uninterrupted sitting.

4.2 Prolonged Sitting

This meta-analysis demonstrated that prolonged uninterrupted sitting leads to a large and significant decline in FMD% (Table 2), specifically in the lower-limbs. The lower-limb specific findings may be explained by several factors. Firstly, the reported differences in the reduction of shear stress during sitting between upper and lower arteries may explain some of the results [4,7–17]. Shear stress is the tangential force created by friction of flowing blood on the luminal surface of all blood vessels and is considered to be a primary regulator of endothelial function [40]. Indeed, multiple lines of evidence demonstrate that reduced shear stress impairs endothelial function [58–63]. Additionally, changes in shear patterns, specifically increases in retrograde shear in the absence of increased antegrade shear, have been shown to blunt FMD responses [64]. Whilst a majority of trials in this analysis only reported mean shear, the limited trials that reported shear patterns consistently found that retrograde shear did not significantly increase in SFA during prolonged sitting [5,17–19]. Instead, the observed reduction in mean shear appears to be the result of decreased antegrade shear [17–19] and overall blood flow, however more research is required across different arteries to confirm this. As a result of low muscle activity in the lower limbs during prolonged sitting, blood flow, and subsequently shear stress, are likely reduced [26]. In contrast, during trials that sampled the BA, participants were allowed to perform desk-based activities throughout the sitting period [6,7,15,18]. Subsequently, reductions in shear stress may not have occurred to the same extent, explaining, in part, the maintenance of BA FMD. Furthermore, by allowing participants to perform desk-based activities, it is unlikely that significant increases in hydrostatic pressure would have occurred within the upper limbs. Conversely, the increased hydrostatic pressure likely experienced by lower limb arteries [65], compounded by the loss of any muscle pump action, may have resulted in blood pooling [26] and activation of the myogenic response, thereby further reducing blood flow-induced shear stress [65].

Secondly, arterial bending created by flexion at the hip and knee joints during sitting may have also impacted vascular function in arteries of the lower limbs. Arterial tortuosity alone has been shown to significantly reduce blood flow and shear stress independent of changes in hydrostatic pressure, whilst also creating an area of turbulent blood flow immediately downstream, and thus resulting in an impaired FMD% [11,66]. The greater decline in FMD% seen at the PA compared to the SFA (Table 2) may be explained by increased turbulent flow, as the assessment site is located close to the knee joint, a site of increased tortuosity. Finally, there is a negative correlation between resting diameters and FMD% [67], and so the greater decline in FMD% in the lower limbs

may be explained by arterial location given that arteries further down the vascular tree become narrower [67]. This is likely true of the present findings whereby the PTA (smallest resting diameter) showed the greatest reduction in FMD% as a consequence of prolonged sitting (Table 2). More studies are required to further investigate this phenomenon, as only one trial assessing the PTA was included in the present analysis. Nevertheless, these data, in tandem with previous work suggesting impaired cerebrovascular endothelial function as a result of prolonged sitting [68], indicate that it is highly conceivable that sitting-induced vascular dysfunction is not solely restricted to larger conduit arteries.

4.3 Sitting Interruption

Despite growing evidence demonstrating leg vascular dysfunction following an acute bout of prolonged sitting, research investigating practical sitting interruption strategies is limited. Our analysis, which included 9 trials and a sample size of 127, demonstrated a significantly (p = 0.01) greater FMD% (WMD = 1.91%, 95% CI: 0.40 to 3.42, SMD = 0.57) when sitting was regularly interrupted compared to uninterrupted sitting.

One of the challenges when investigating the effect of interrupting sitting is the variety of arteries assessed. It is apparent that lower limb arteries are more affected by uninterrupted sitting, and therefore perhaps also more amenable to the effects of interrupting sitting periods. Indeed, subgroup analysis demonstrated that BA FMD% was the least affected by interruption. Conversely, whilst failing to reach significance, sitting interruption had a moderate effect on SFA FMD% and PA FMD% (Table 3). The greater FMD% observed in lower limb arteries following interruption is likely the result of preserved blood flow and subsequently shear stress as a product of greater lower-extremity activity [10]. In order to identify optimum interruption strategies to preserve vascular function, separate subgroup analysis was performed.

Findings from the interruption subgroup analysis indicated that both simple resistance activities and aerobic interruption strategies resulted in moderate non-significant differences in FMD% between the experimental and control conditions (Table 3). The failure of any of the subgroups to reach significance may be a product of the limited number of trials, or the observed heterogeneity present within each subgroup as a product of differing FMD assessment locations or experimental designs. Indeed, this is apparent in the simple resistance activities subgroup which only consisted of 3 trials, 2 of which assessed BA FMD% [6,7] and the third assessed SFA FMD% [7]. It is plausible that simple resistance activities may preserve vascular function, however more trials assessing lower limb arteries are necessary.

With respect to the aerobic subgroup, whilst failing to reach statistical significance, it is likely that this modality is a viable interruption strategy. Indeed, of the 5 trials within the subgroup 4 trials reported an improvement in

FMD% from baseline [5,10,17] and 3 reported improvements between conditions [5,10,17]. The considerable heterogeneity within this subgroup (I^2 = 86%) likely contributed to the lack of statistical significance and may be a result of key methodological differences between trials. Of particular note are the findings by Carter et al. [5], which shows that the aerobic interruption strategy utilised resulted in a poorer FMD% outcome than the control condition (Figure 4). However, this may be a result of uncontrolled lower limb movement during the control condition. In an attempt to improve ecological validity, Carter et al. [5] was the only trial to not restrict lower limb movement during the control condition and may explain why it is the only trial to show improved FMD% in a lower limb artery in response to prolonged sitting (Figure 2). Subsequently, whilst the original data from this trial shows that the aerobic interruption strategy preserved vascular function, it is masked in this analysis by the elevated control FMD%. Further supporting the notion that aerobic interruption strategies may be beneficial in preventing sitting-induced vascular dysfunction, McManus et al. [69] demonstrated that aerobic interruptions could prevent sitting-induced leg vascular dysfunction in 9-year old girls. As the inclusion criteria for the current meta-analysis was adults, these data were not included in the current analysis. However, this finding, in combination with the data from the present meta-analysis, indicates that aerobic interruption strategies may prevent sitting-induced vascular dysfunction.

Finally, whilst standing has been suggested as a viable sitting interruption strategy and can prevent a decline in central arterial health during bouts of prolonged sitting [70], the present meta-analysis revealed a non-significant trivial difference in FMD% in the lower limbs (WMD = 0.24, 95% CI: -0.91 to 1.38, SMD = 0.16) between conditions. It is possible that standing breaks are an insufficient stimulus to increase shear stress and thus prevent sitting-induced vascular dysfunction. However, it is noteworthy that when sitting is fully substituted by standing for 3 hours, leg vascular function is effectively preserved [11]. Accordingly, it appears that while standing breaks may not be sufficient to prevent sitting-induced leg vascular dysfunction, replacing sitting for standing could be a viable strategy to retain vascular function; yet further research is needed to support this conclusion.

4.4 Methodological concerns

Determining the effect of interrupting sitting is made challenging by the differences in the experimental design and protocols used by the included trials. This may also be the cause of the high heterogeneity present in the separate subgroup analyses (Table 3). For example, the considerable heterogeneity across PA trials (I^2 = 90%) may be explained by key differences in the time between the end of the final interruption and the final FMD

assessment, which ranged from ~10 [10] to ~60 [9] minutes. Given that shear stress, the principal driver of changes in FMD, has been shown to significantly decrease following as little as 10 minutes of sitting [71], extended periods of inactivity (i.e., 60 minutes) prior to post-sitting FMD assessments will likely mask the true effect of interruption strategies. Conversely, by assessing FMD within 10 minutes of the final interruption [10] it could be argued that the subsequent elevation in shear stress as a consequence of the interruption will likely mask the true effect of prolonged sitting [4]. Whilst it is beyond the scope of this article to suggest an optimum methodology, it is clear that the differences between trials seeking to answer similar questions make drawing conclusions challenging. The development and implementation of standardised guidelines may facilitate a better understanding of this research area.

4.5 Implications

A summary of the implications is provided in Table 4. Prior to this review, growing evidence suggested that bouts of prolonged sitting may negatively impact vascular function and that regular interruptions to sitting may offset that effect. However, the magnitude of the effect of sitting on vascular function and whether effects differed across arteries was unclear. Additionally, whilst various studies have investigated potential interruption strategies, until now, it remained uncertain how different arteries were affected and whether an optimum interruption strategy may exist. The current study is the first to consolidate the existing data in this area. The data indicate that bouts of prolonged sitting ($\geq$1hr) result in significant, moderate to large declines in FMD% in lower limb arteries, but not in the BA. Further to this, whilst current data is unable to determine an optimum interruption strategy aimed at preventing vascular dysfunction, aerobic interruption strategies may offer the most robust protective effect, likely as a result of increased blood flow-induced shear stress.

Several important gaps in the literature were identified. Given the low number of included trials investigating interruption strategies, the current meta-analysis was unable to determine the optimal dose, duration, and intensity of sitting interruption. Also, there is currently a discourse in the methodologies employed across studies in this area, with one example being the posture in which FMD is assessed. Current FMD guidelines state that assessments are performed with participants supine [42]. However, 13 of the 22 trials in this analysis assessed FMD with participants seated or semi-recumbent [4,7,13,15,17–20]. To date, there is no evidence that this is either

Table 4 Summary of findings and implications

What did we know prior to this study?
- Epidemiological data suggests a link between cardiovascular disease incidence and time spent sitting. - Bouts of acute prolonged sitting can result in vascular dysfunction, a precursor to CVD. - Sitting interruption strategies may offset vascular dysfunction.
What did we not know prior to this study?

- Extent to which prolonged sitting affects vascular function in upper and lower limb arteries. - How interrupted sitting affects vascular function compared to uninterrupted sitting. - The optimum interruption strategy to preserve vascular function during prolonged sitting.
What does this study add?
- Prolonged sitting results in a significant decline in vascular function in lower- but not upper-limb arteries. - Interrupting prolonged sitting with aerobic activities may preserve vascular function. - Identification of inconsistencies in the methods employed within this research area.
How do we use this new information?
- Future research should focus on assessing lower limb arteries only. - Simple resistance activities or aerobic interruption strategies may be viable means of preserving vascular function during exposure to prolonged sitting.
What needs to happen next to move the field forward?
- Standardised guidelines should be designed and implemented for this research area. - Optimal dose and frequency of interruption should be determined. - Findings from laboratory-based sitting studies presented herein should be confirmed in more real-life (i.e., ecological) scenarios.

an accurate (valid) or reliable measure compared to the recommended supine position. Additionally, in trials that conducted FMD assessments with participants in a supine position, some trials have reported performing FMD assessments immediately [10], whereas others imply slightly longer rest periods [8]. These divergent practices, post-sitting transition are likely to increase the risk of under- or over-estimation of FMD. The development and implementation of standardised guidelines may improve the congruency of future research. Additionally, the validation of seated FMD assessments would allow researchers to confidently chart the time course of vascular dysfunction as a result of prolonged uninterrupted sitting and whether a dose-response curve exists. Currently, trials that have performed seated SFA FMD assessments multiple times throughout a bout of uninterrupted sitting have all demonstrated significant declines within the first hour of sitting [4,17–19]. However, some have proceeded to continue a gradual decline as sitting time increases [4], whereas others have shown an upwards trend past the 1 hour point [7,17–19]. Without a validated means of assessing vascular function with participants in a seated position and standardised guidelines, understanding the time course of dysfunction and the mechanisms responsible remain challenging.

5 Conclusions

Epidemiological literature has established a positive association between sedentary behaviours, such as prolonged sitting, and CVD incidence and all-cause mortality. Vascular dysfunction may be a key mechanism in explaining this association. This meta-analysis is the first to amalgamate the existing data of the effect of prolonged sitting on vascular function. The results of this analysis indicate that (1) periods of prolonged uninterrupted sitting in excess of 1 hour may lead to a meaningful decrease in vascular function in lower limb arteries, and that, (2) this dysfunction can be avoided by regularly interrupting sitting, particularly with aerobic interruptions or simple

resistance activities. In order to identify optimum interruption strategies, future research, utilising synergistic experimental methodologies is required. This future research should aim to determine the optimal dose, duration, and intensity of sitting interruption.

Data Availability

The data analysed for this meta-analysis are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Funding: No sources of funding were used to assist in the preparation of this article

Conflicts of Interest: Craig Paterson, Simon Fryer, Gabriel Zieff, Keeron Stone, Daniel Credeur, Bethany Barone Gibbs, Jaume Padilla, John Parker, and Lee Stoner declare that they have no conflicts of interest relevant to the content of this review.

Authorship Contributions: CP and GZ completed the literature search. CP, SF, and LS completed quality assessment. CP and GZ extracted all relevant data from selected articles. CP and LS conducted data analysis. CP, SF, KS, and LS wrote the first draft of the manuscript. GZ, BBG, JP, and JP revised the original manuscript. All authors read and approved the final manuscript.

References

1. Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, et al. Sedentary behavior research network (SBRN)–terminology consensus project process and outcome. Int J Behav Nutr Phys Act. 2017;14:75.

2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics - 2019 update: A report from the American Heart Association. Circulation. 2019;139:e56–528.

3. Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. Ann Intern Med. 2015;162:123–32.

4. Ballard KD, Duguid RM, Berry CW, Dey P, Bruno RS, Ward RM, et al. Effects of prior aerobic exercise on sitting-induced vascular dysfunction in healthy men. Eur J Appl Physiol. 2017;117:2509–18.

5. Carter SE, Draijer R, Holder SM, Brown L, Thijssen DH, Hopkins ND. Effect of different walking break strategies on superficial femoral artery endothelial function. Physiol Rep. 2019;7:e14190.

6. Carter SE, Gladwell VF. Effect of breaking up sedentary time with callisthenics on endothelial function. J Sports Sci. 2017;35:1508–14.

7. Climie RE, Wheeler MJ, Grace M, Lambert E, Cohen N, Owen N, et al. Simple intermittent resistance activity mitigates the detrimental effect of prolonged unbroken sitting on arterial function in overweight and obese adults. J Appl Physiol. 2018;125:1787–94.

8. Credeur DP, Miller SM, Jones R, Lee S, Dolbow DR, Fryer SM, et al. Impact of prolonged sitting on peripheral and central vascular health. Am J Cardiol. 2018;123:260–6.

9. Kruse NT, Hughes WE, Benzo RM, Carr LJ, Casey DP. Workplace strategies to prevent sitting-induced endothelial dysfunction. Med Sci Sports Exerc. 2018;50:801–8.

10. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, Padilla J. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. Am J Physiol-Heart Circ Physiol. 2016;311:H177–82.

11. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Padilla J. Prior exercise and standing as strategies to circumvent sitting-induced leg endothelial dysfunction. Clin Sci. 2017;131:1045–53.

12. Morishima T, Tsuchiya Y, Padilla J, Ochi E. Eight weeks of fish oil supplementation does not prevent sitting-induced leg endothelial dysfunction. Appl Physiol Nutr Metab. 2019;1–6.

13. O'Brien MW, Johns JA, Williams TD, Kimmerly DS. Sex does not influence impairments in popliteal endothelial-dependent vasodilator or vasoconstrictor responses following prolonged sitting. J Appl Physiol. 2019;127:679–87.

14. Padilla J, Sheldon RD, Sitar DM, Newcomer SC. Impact of acute exposure to increased hydrostatic pressure and reduced shear rate on conduit artery endothelial function: a limb-specific response. Am J Physiol-Heart Circ Physiol. 2009;297:H1103–8.

15. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro-and macrovascular dilator function. Exp Physiol. 2015;100:829–38.

16. Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ, et al. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. Am J Physiol-Heart Circ Physiol. 2016;310:H648–53.

17. Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of prolonged sitting and breaks in sitting time on endothelial function. Med Sci Sports Exerc. 2015;47:843–9.

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

19. Thosar SS, Bielko SL, Wiggins CS, Klaunig JE, Mather KJ, Wallace JP. Antioxidant vitamin C prevents decline in endothelial function during sitting. Med Sci Monit Int Med J Exp Clin Res. 2015;21:1015–21.

20. Vranish JR, Young BE, Kaur J, Patik JC, Padilla J, Fadel PJ. Influence of sex on microvascular and macrovascular responses to prolonged sitting. Am J Physiol-Heart Circ Physiol. 2017;312:H800–5.

21. Lima BB, Hammadah M, Kim JH, Uphoff I, Shah A, Levantsevych O, et al. Association of transient endothelial dysfunction induced by mental stress with major adverse cardiovascular events in men and women with coronary artery disease. JAMA Cardiol. 2019;4:988–96.

22. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010;26:631–40.

23. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: A systematic review with meta-analysis. Int J Cardiol. 2013;168:344–51.

24. Xu Y, Arora RC, Hiebert BM, Lerner B, Szwajcer A, McDonald K, et al. Non-invasive endothelial function testing and the risk of adverse outcomes: a systematic review and meta-analysis. Eur Heart J - Cardiovasc Imaging. 2014;15:736–46.

25. Matsuzawa Y, Kwon T, Lennon RJ, Lerman LO, Lerman A. Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. J Am Heart Assoc. 2015;4:e002270.

26. Padilla J, Fadel PJ. Prolonged sitting leg vasculopathy: contributing factors and clinical implications. Am J Physiol-Heart Circ Physiol. 2017;313:H722–8.

27. Bauman A, Ainsworth BE, Sallis JF, Hagströmer M, Craig CL, Bull FC, et al. The descriptive epidemiology of sitting: a 20-country comparison using the International Physical Activity Questionnaire (IPAQ). Am J Prev Med. 2011;41:228–35.

28. Clemes SA, Houdmont J, Munir F, Wilson K, Kerr R, Addley K. Descriptive epidemiology of domain-specific sitting in working adults: the Stormont Study. J Public Health. 2015;38:53–60.

29. Clemes SA, O'connell SE, Edwardson CL. Office workers' objectively measured sedentary behavior and physical activity during and outside working hours. J Occup Environ Med. 2014;56:298–303.

30. Healy GN, Wijndaele K, Dunstan DW, Shaw JE, Salmon J, Zimmet PZ, et al. Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). Diabetes Care. 2008;31:369–71.

31. Jans MP, Proper KI, Hildebrandt VH. Sedentary behavior in Dutch workers: differences between occupations and business sectors. Am J Prev Med. 2007;33:450–4.

32. Mabry R, Koohsari MJ, Bull F, Owen N. A systematic review of physical activity and sedentary behaviour research in the oil-producing countries of the Arabian Peninsula. BMC Public Health. 2016;16:1003.

33. Matthews CE, Chen KY, Freedson PS, Buchowski MS, Beech BM, Pate RR, et al. Amount of Time Spent in Sedentary Behaviors in the United States, 2003–2004. Am J Epidemiol. 2008;167:875–81.

34. Thorp AA, Healy GN, Winkler E, Clark BK, Gardiner PA, Owen N, et al. Prolonged sedentary time and physical activity in workplace and non-work contexts: a cross-sectional study of office, customer service and call centre employees. Int J Behav Nutr Phys Act. 2012;9:128.

35. Win AM, Yen LW, Tan KH, Lim RBT, Chia KS, Mueller-Riemenschneider F. Patterns of physical activity and sedentary behavior in a representative sample of a multi-ethnic South-East Asian population: a cross-sectional study. BMC Public Health. 2015;15:318.

36. Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLOS Med. 2009;6:e1000097.

37. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions. Chichester: Wiley; 2008.

38. Heyland DK, MacDonald S, Keefe L, Drover JW. Total parenteral nutrition in the critically ill patient: a meta-analysis. JAMA. 1998;280:2013–9.

39. Heyland DK, Novak F, Drover JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients?: A systematic review of the evidence. JAMA. 2001;286:944–53.

40. Stoner L, Sabatier MJ. Use of ultrasound for non-invasive assessment of flow-mediated dilation. J Atheroscler Thromb. 2012;19:407–21.

41. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol-Heart Circ Physiol. 2011;300:H2–12.

42. Thijssen DH, Bruno RM, van Mil AC, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J. 2019;40:2534–47.

43. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. Eur Heart J. 2004;26:363–8.

44. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods. 2010;1:97–111.

45. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557–60.

46. Furuya-Kanamori L, Barendregt JJ, Doi SA. A new improved graphical and quantitative method for detecting bias in meta-analysis. Int J Evid Based Healthc. 2018;16:195–203.

47. Stoner L, Tarrant MA, Fryer S, Faulkner J. How should flow-mediated dilation be normalized to its stimulus? Clin Physiol Funct Imaging. 2013;33:75–8.

48. Stoner L, Faulkner J, Sabatier MJ. Is allometric scaling really a panacea for flow-mediated dilation? Commentary on paper by Atkinson and Batterham. Atherosclerosis. 2013;228:280–281.

49. McLay KM, Nederveen JP, Koval JJ, Paterson DH, Murias JM. Allometric scaling of flow-mediated dilation: is it always helpful? Clin Physiol Funct Imaging. 2018;38:663–9.

50. Harris RA, Padilla J. Proper "normalization" of flow-mediated dilation for shear. J Appl Physiol. 2007;103:1108–1108.

51. 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. 2013;18:354–65.

52. Atkinson G. Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas. 2014;35:1825–35.

53. Williams MRI, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, et al. Variations in endothelial function and arterial compliance during the menstrual cycle. J Clin Endocrinol Metab. 2001;86:5389–95.

54. Adkisson EJ, Casey DP, Beck DT, Gurovich AN, Martin JS, Braith RW. Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. Exp Biol Med. 2010;235:111–8.

55. Harris RA, Tedjasaputra V, Zhao J, Richardson RS. Premenopausal women exhibit an inherent protection of endothelial function following a high-fat meal. Reprod Sci. 2012;19:221–8.

56. Hashimoto Masayoshi, Akishita Masahiro, Eto Masato, Ishikawa Michiro, Kozaki Koichi, Toba Kenji, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation. 1995;92:3431–5.

57. Shenouda N, Priest SE, Rizzuto VI, MacDonald MJ. Brachial artery endothelial function is stable across a menstrual and oral contraceptive pill cycle but lower in premenopausal women than in age-matched men. Am J Physiol-Heart Circ Physiol. 2018;315:H366–74.

58. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. J Physiol. 2005;568:357–69.

59. Cheng C, de Crom R, van Haperen R, Helderman F, Gourabi BM, van Damme LC, et al. The role of shear stress in atherosclerosis. Cell Biochem Biophys. 2004;41:279–94.

60. Cheng C, Tempel D, Van Haperen R, Van Der Baan A, Grosveld F, Daemen M, et al. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation. 2006;113:2744–53.

61. Cheng JL, Au JS, MacDonald MJ. Peripheral artery endothelial function responses to altered shear stress patterns in humans. Exp Physiol. 2019;104:1126–35.

62. Green DJ, Hopman MT, Padilla J, Laughlin MH, Thijssen DH. Vascular adaptation to exercise in humans: role of hemodynamic stimuli. Physiol Rev. 2017;97:495–528.

63. Ridger V, Krams R, Carpi A, Evans PC. Hemodynamic parameters regulating vascular inflammation and atherosclerosis: a brief update. Biomed Pharmacother. 2008;62:536–40.

64. Thijssen DH, Dawson EA, Tinken TM, Cable NT, Green DJ. Retrograde flow and shear rate acutely impair endothelial function in humans. Hypertension. 2009;53:986–92.

65. Kitano A, Shoemaker JK, Ichinose M, Wada H, Nishiyasu T. Comparison of cardiovascular responses between lower body negative pressure and head-up tilt. J Appl Physiol. 2005;98:2081–6.

66. Walsh LK, Restaino RM, Martinez-Lemus LA, Padilla J. Prolonged leg bending impairs endothelial function in the popliteal artery. Physiol Rep. 2017;5:e13478.

67. 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. 2008;295:H1927–34.

68. Carter SE, Draijer R, Holder SM, Brown L, Thijssen DH, Hopkins ND. Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. J Appl Physiol. 2018;125:790–8.

69. McManus AM, Ainslie PN, Green DJ, Simair RG, Smith K, Lewis N. Impact of prolonged sitting on vascular function in young girls. Exp Physiol. 2015;100:1379–87.

70. Barone Gibbs B, Kowalsky RJ, Perdomo SJ, Taormina JM, Balzer JR, Jakicic JM. Effect of alternating standing and sitting on blood pressure and pulse wave velocity during a simulated workday in adults with overweight/obesity. J Hypertens. 2017;35:2411–8.

71. Vranish JR, Young BE, Stephens BY, Kaur J, Padilla J, Fadel PJ. Brief periods of inactivity reduce leg microvascular, but not macrovascular, function in healthy young men. Exp Physiol. 2018;103:1425–34.

Electronic Supplementary Material

Electronic Supplementary Material Appendix S1 Modified Heyland Methodological Quality Score assessment criteria