

The Effects of Acute Exposure to Prolonged Sitting, With and Without Interruption, on Vascular Function Among Adults: A Meta-analysis

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Published in:
Sports Medicine

Publication date:
2020

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[10.1007/s40279-020-01325-5](https://doi.org/10.1007/s40279-020-01325-5)

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Citation for published version (APA):

Paterson, C., Fryer, S., Zieff, G., Stone, K., Credeur, D. P., Barone Gibbs, B., Padilla, J., Parker, J. K., & Stoner, L. (2020). The Effects of Acute Exposure to Prolonged Sitting, With and Without Interruption, on Vascular Function Among Adults: A Meta-analysis. *Sports Medicine*, *50*(11), 1929-1942. <https://doi.org/10.1007/s40279-020-01325-5>

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

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Conflicts of interest: None

Source of funding: None

1 **Abstract**

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

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

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

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

20 Appraisal and synthesis methods: 1776 articles were identified, of which 17 (22 trials, $n=269$) met inclusion
21 criteria for objective (1). Of those, 17 articles (9 trials, $n=127$) met the inclusion criteria for objective (2).

22 Weighted mean differences (WMD), 95% confidence intervals (95% CI), and standardised mean difference
23 (SMD) were calculated for all trials using random-effects meta-analysis modelling. SMD was used to determine
24 the magnitude of effect, where <0.2 , 0.2, 0.5, and 0.8 was defined as trivial, small, moderate, and large
25 respectively.

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

29 resulted in a significantly higher FMD% compared to uninterrupted sitting (WMD=1.91%, 95% CI:0.40, 3.42,
30 SMD=0.57). Subgroup analyses failed to identify an optimum interruption strategy but revealed moderate non-
31 significant effects for aerobic interventions (WMD=2.17%, 95% CI:-0.34 to 4.67, SMD=0.69) and simple
32 resistance activities (WMD=2.40%, 95% CI:-0.08 to 4.88, SMD=0.55) and a trivial effect for standing
33 interruptions (WMD=0.24%, 95% CI:-0.90 to 1.38, SMD=0.16).

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

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39 **Conflicts of interest:** None

40 **Source of funding:** None

41

42 **Key Points**

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

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

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

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59 **1.1 Introduction**

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

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

81 **1.2 Objectives**

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

88 **2 Methods**

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

91 **2.1 Data Sources and Searches**

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

99 **2.2 Article Selection**

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

115 **2.3 Data Extraction and Quality Assessment**

116 Data extracted for each eligible trial included bibliographic information (author, publication year), collected
117 measures, sample characteristics (age, sex, body mass index, etc.), details of any interventions, arterial site, and
118 uncorrected relative FMD values. Uncorrected FMD values were collected, as opposed to allometrically scaled or

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

133 2.4 Data Synthesis

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

140 2.5 Data Analysis

141 All extracted data were entered into software specifically designed for meta-analyses (MetaXL,
142 http://www.epigear.com/index_files/metaxl.html). Outcome measures were calculated as weighted mean
143 differences (WMDs) as well as the standardised mean difference (SMD). The SMD was used to determine the
144 magnitude of the effect, where <0.2, 0.2, 0.5, and 0.8 was defined as trivial, small, moderate, and large respectively
145 [42]. Random-effects modelling, with the DerSimonian-Laird method, was used for both analyses as it allows for
146 heterogeneity in experimental procedures and accounts for both within- and between-trial variance [44]. The
147 statistical heterogeneity across trials included in the meta-analysis was assessed using the I^2 statistic, where <25%,
148 25%, and 75% represent low, moderate, and considerable heterogeneity, respectively [45]. Sensitivity analyses

149 were performed by excluding one trial at a time to test the robustness of the pooled results. The Luis Furuya-
150 Kanamori (LFK) index was used as it is a means of identifying and quantifying asymmetry and potential small
151 study bias, where <1 indicates no asymmetry, 1 to 2 suggests minor asymmetry, and >2 indicates major asymmetry
152 [46]. Additionally, publication bias was evaluated by visual inspection of the Begg's funnel plot when (i) at least
153 10 trials were included in the meta-analysis, and (ii) there was substantial variation in sample size for the included
154 trials [37]. Two authors (LS and CP) conducted the data analysis.

155 **3 Results**

156 **3.1 Literature Search and Trial Selection**

157 The literature search strategy is outlined in Figure 1. Initial database searches identified a total of 1797 potentially
158 eligible articles with a further 5 identified through manual searches. Following screening of titles and abstracts,
159 1769 articles were excluded because they did not meet inclusion criteria. The remaining 33 papers underwent full
160 text screening and 16 further studies were excluded. The final analysis included 17 studies (22 trials) for objective
161 (1), of which 6 (9 trials) were included in a separate analysis of interruptions to prolonged sitting for objective
162 (2).

163 **3.2 Characteristics of Included Studies**

164 The trial characteristics are summarised in Table 1. The number of participants in each trial ranged from 8 [14] –
165 20 [8]. Of the 22 trials, 12 included only male participants [4,12–20], and 2 included only females [13,20], with
166 8 trials included both sexes [5–8,10,11]. Bouts of prolonged sitting ranged from 1.5 [6] to 6 hours [15], with a
167 modal sitting duration of 3 [4,10–14,16–20]. Assessments of FMD were carried out predominantly in the lower
168 limb, with only 4 of the 18 trials assessing brachial artery (BA) FMD [6,7,15,18]. Of the trials that assessed FMD
169 in the lower limb, 6 assessed the superficial femoral artery (SFA) [4,5,7,17–19], and 11 assessed the popliteal
170 artery (PA) [9–15,20], with 1 assessing the posterior tibial artery (PTA) [8]. Of the 18 trials, 9 included strategies
171 to interrupt the bout of prolonged sitting [5–7,9,10,17]. These interruptions were categorised as aerobic
172 [5,9,10,17], simple resistance activities [6,7], or standing [9].

173 **3.3 Methodological Quality Assessment**

174 The methodological assessment of included trials is summarised in Table 1. The quality of studies ranged from 3
175 to 8 out of a possible maximum of 10, with the median quality score being 7. All trials assessed and reported all
176 collected data and reported any dropouts or unusable data. For blinding, 10 trials reported that offline analysis of
177 FMD videos was performed by a blinded technician/researcher [4,7,13,14,17–19]. Ten trials reported the

178 published FMD guidelines that they adhered to [5,6,8,11,13,17–19], and 8 trials performed FMD with participants
179 in the suggested supine position [5,6,8–12,16].

180 3.4 Synthesis of the Results

181 3.4.1 Effects of Prolonged Sitting on Flow-Mediated Dilatation

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

191 3.4.2 Effects of Sitting Interruption on the Flow-Mediated Dilatation Response to Prolonged Sitting

192 Across sitting interruption strategies there was a moderate, significantly greater FMD% for the experimental
193 (interrupted) conditions compared to the control (uninterrupted) (WMD = 1.91%, 95% CI: 0.40 to 3.42, $p = 0.01$,
194 SMD = 0.57) (Figure 4). Sensitivity analysis indicated that removal of any one of 3 trials [7,10,17] resulted in a
195 reduced overall effect but did not result in a loss of statistical significance. An LFK index of 3.36 indicated major
196 asymmetry. The heterogeneity for this analysis was considerable ($I^2 = 79%$, $p < 0.001$) and may be explained by
197 the low number of trials, testing FMD on different arteries, and the use of varying interruption strategies. With
198 respect to different arteries, subgroup analysis revealed non-significant effects on BA, SFA, and PA FMD%
199 (Table 3). This analysis also revealed considerable heterogeneity in the SFA and PA subgroups ($I^2 = 77%$ and
200 90% respectively). With regards to sitting interruption strategies, simple resistance activities and aerobic
201 interruption strategies resulted in non-significant moderately greater FMD% compared to control conditions
202 (Table 3). This subgroup analysis also revealed considerable heterogeneity for the aerobic subgroup ($I^2 = 86%$)
203 and moderate heterogeneity for the simple resistance activities subgroup ($I^2 = 47%$). Finally, only one trial
204 included standing as an interruption strategy [9], reporting a non-significant difference in FMD% between
205 conditions (WMD = 0.24, 95% CI: -0.90 to 1.38) (Table 3).

206 4 Discussion

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

213 4.1 Limitations

214 Whilst this meta-analysis has produced meaningful information, several potential limitations should be
215 acknowledged when interpreting the results of this analysis. Firstly, there was a limited number of eligible trials
216 and the sample sizes were small (range = 8 – 20, median = 12). Additionally, only 4 trials reported sample size
217 calculation for the FMD outcome [4,16,17,19]. However, this is the first meta-analysis looking at the effects of
218 prolonged sitting with and without interruption and some important methodological insights for future studies are
219 noted. Second, our analysis considered change in FMD%. FMD% is a ratio calculated by dividing the maximum
220 change in artery diameter in response to reactive hyperaemia by the resting artery diameter. This approach has
221 been criticised as the change in diameter is inversely proportional to baseline diameter and thus a ratio is
222 statistically unsuitable [47]. Allometric scaling has been offered as a means of controlling for the influence of
223 baseline diameter, however this approach may not be able to adequately correct FMD% in different vascular beds
224 or at an individual level, i.e. it can only be applied to group means [42,48,49]. An alternate approach has been to
225 correct FMD% by using shear rate as a covariate [50,51], though this approach has also been suggested to have
226 limitations [52]. These points, taken together, indicate that there is a current lack of consensus about the best
227 statistical strategy for controlling for factors that influence FMD. Consequently, current FMD guidelines still
228 suggest reporting FMD% irrespective of any further analysis [40,41]. Subsequently, these data are readily
229 available within the literature and, whilst certain limitations of the metric are acknowledged, FMD% served as an
230 appropriate metric for this analysis. Thirdly, this study has highlighted a sex-specific void in the research.
231 Specifically, 75% of the overall sample were male, and of the 10 trials which included females, 6 studied females
232 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
233 studied menopausal women [7]. Given the potential influence of the menstrual cycle on FMD% [53–57], and the
234 small number of females sampled, generalising the findings of this meta-analysis to females is difficult. Lastly,
235 this analysis only considered the difference between baseline and final FMD%, so any inferences regarding the
236 time course of vascular dysfunction during uninterrupted sitting cannot be made. This practice was based on ~70%

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

240 4.2 Prolonged Sitting

241 This meta-analysis demonstrated that prolonged uninterrupted sitting leads to a large and significant decline in
242 FMD% (Table 2), specifically in the lower-limbs. The lower-limb specific findings may be explained by several
243 factors. Firstly, the reported differences in the reduction of shear stress during sitting between upper and lower
244 arteries may explain some of the results [4,7–17]. Shear stress is the tangential force created by friction of flowing
245 blood on the luminal surface of all blood vessels and is considered to be a primary regulator of endothelial function
246 [40]. Indeed, multiple lines of evidence demonstrate that reduced shear stress impairs endothelial function [58–
247 63]. Additionally, changes in shear patterns, specifically increases in retrograde shear in the absence of increased
248 antegrade shear, have been shown to blunt FMD responses [64]. Whilst a majority of trials in this analysis only
249 reported mean shear, the limited trials that reported shear patterns consistently found that retrograde shear did not
250 significantly increase in SFA during prolonged sitting [5,17–19]. Instead, the observed reduction in mean shear
251 appears to be the result of decreased antegrade shear [17–19] and overall blood flow, however more research is
252 required across different arteries to confirm this. As a result of low muscle activity in the lower limbs during
253 prolonged sitting, blood flow, and subsequently shear stress, are likely reduced [26]. In contrast, during trials that
254 sampled the BA, participants were allowed to perform desk-based activities throughout the sitting period
255 [6,7,15,18]. Subsequently, reductions in shear stress may not have occurred to the same extent, explaining, in part,
256 the maintenance of BA FMD. Furthermore, by allowing participants to perform desk-based activities, it is unlikely
257 that significant increases in hydrostatic pressure would have occurred within the upper limbs. Conversely, the
258 increased hydrostatic pressure likely experienced by lower limb arteries [65], compounded by the loss of any
259 muscle pump action, may have resulted in blood pooling [26] and activation of the myogenic response, thereby
260 further reducing blood flow-induced shear stress [65].

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

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

275 4.3 Sitting Interruption

276 Despite growing evidence demonstrating leg vascular dysfunction following an acute bout of prolonged sitting,
277 research investigating practical sitting interruption strategies is limited. Our analysis, which included 9 trials and
278 a sample size of 127, demonstrated a significantly ($p = 0.01$) greater FMD% (WMD = 1.91%, 95% CI: 0.40 to
279 3.42, SMD = 0.57) when sitting was regularly interrupted compared to uninterrupted sitting.

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

288 Findings from the interruption subgroup analysis indicated that both simple resistance activities and aerobic
289 interruption strategies resulted in moderate non-significant differences in FMD% between the experimental and
290 control conditions (Table 3). The failure of any of the subgroups to reach significance may be a product of the
291 limited number of trials, or the observed heterogeneity present within each subgroup as a product of differing
292 FMD assessment locations or experimental designs. Indeed, this is apparent in the simple resistance activities
293 subgroup which only consisted of 3 trials, 2 of which assessed BA FMD% [6,7] and the third assessed SFA FMD%
294 [7]. It is plausible that simple resistance activities may preserve vascular function, however more trials assessing
295 lower limb arteries are necessary.

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

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

321 4.4 Methodological concerns

322 Determining the effect of interrupting sitting is made challenging by the differences in the experimental design
323 and protocols used by the included trials. This may also be the cause of the high heterogeneity present in the
324 separate subgroup analyses (Table 3). For example, the considerable heterogeneity across PA trials ($I^2 = 90\%$)

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

335 4.4 Implications

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

346 Several important gaps in the literature were identified. Given the low number of included trials investigating
347 interruption strategies, the current meta-analysis was unable to determine the optimal dose, duration, and intensity
348 of sitting interruption. Also, there is currently a discourse in the methodologies employed across studies in this
349 area, with one example being the posture in which FMD is assessed. Current FMD guidelines state that
350 assessments are performed with participants supine [42]. However, 13 of the 22 trials in this analysis assessed
351 FMD with participants seated or semi-recumbent [4,7,13,15,17–20]. To date, there is no evidence that this is either
352 an accurate (valid) or reliable measure compared to the recommended supine position. Additionally, in trials that
353 conducted FMD assessments with participants in a supine position, some trials have reported performing FMD
354 assessments immediately [10], whereas others imply slightly longer rest periods [8]. These divergent practices,

355 post-sitting transition are likely to increase the risk of under- or over-estimation of FMD. The development and
356 implementation of standardised guidelines may improve the congruency of future research. Additionally, the
357 validation of seated FMD assessments would allow researchers to confidently chart the time course of vascular
358 dysfunction as a result of prolonged uninterrupted sitting and whether a dose-response curve exists. Currently,
359 trials that have performed seated SFA FMD assessments multiple times throughout a bout of uninterrupted sitting
360 have all demonstrated significant declines within the first hour of sitting [4,17–19]. However, some have
361 proceeded to continue a gradual decline as sitting time increases [4], whereas others have shown an upwards trend
362 past the 1 hour point [7,17–19]. Without a validated means of assessing vascular function with participants in a
363 seated position and standardised guidelines, understanding the time course of dysfunction and the mechanisms
364 responsible remain challenging.

365 **5 Conclusions**

366 Epidemiological literature has established a positive association between sedentary behaviours, such as prolonged
367 sitting, and CVD incidence and all-cause mortality. Vascular dysfunction may be a key mechanism in explaining
368 this association. This meta-analysis is the first to amalgamate the existing data of the effect of prolonged sitting
369 on vascular function. The results of this analysis indicate that (1) periods of prolonged uninterrupted sitting in
370 excess of 1 hour may lead to a meaningful decrease in vascular function in lower limb arteries, and that, (2) this
371 dysfunction can be avoided by regularly interrupting sitting, particularly with aerobic interruptions or simple
372 resistance activities. In order to identify optimum interruption strategies, future research, utilising synergistic
373 experimental methodologies is required. This future research should aim to determine the optimal dose, duration,
374 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.

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Table Legends

Table 1 Characteristics of the included trials

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

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

Table 4 Summary of findings and implications

Figure Legends

Fig. 1 Flow chart presenting the inclusion and exclusion criteria

Fig. 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.

Fig. 3 Funnel plot for uninterrupted sitting meta-analysis.

Fig. 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.

Electronic Supplementary Material

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

Ref	Quality	Sample [n (F); mean		FMD assessment site	Sitting duration (h)	Interruption strategy
		age, years (SD)]				
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.

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.

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.

What did we know prior to this study?
<ul style="list-style-type: none"> - 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?
<ul style="list-style-type: none"> - 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?
<ul style="list-style-type: none"> - 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?
<ul style="list-style-type: none"> - 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?
<ul style="list-style-type: none"> - 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.







