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Taylor, F. C., Pinto, A. J., Maniar, N., Dunstan, D. W. and Green, D. J. (2022). The acute effects of prolonged uninterrupted sitting on vascular function : A systematic review and meta-analysis. *Medicine and Science in Sports and Exercise,* 54(1), pp. 67-76. https://doi.org/10.1249/MSS.0000000000002763

Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

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Accepted for Publication: 15 July 2021

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000002763

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This research was not supported by any grants. No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. All authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

ABSTRACT

Objective To determine the dose-response relationship between prolonged sitting and vascular function in healthy individuals and those with metabolic disturbances. To investigate the acute effects, on vascular function, of interventions that target interrupting prolonged sitting. **Design** Systematic review with meta-analysis.

Data sources Ovid Embase, Ovid Medline, PubMed, and CINAHL were searched from inception to 4 December 2020.

Eligibility criteria Randomised crossover trials, quasi-randomised trials, and parallel group trials where vascular function (flow-mediated dilation, FMD) was assessed before and after an acute period of sedentary behaviour.

Results Prolonged sitting resulted in a significant decrease in the standardised mean change (SMC) for lower-limb FMD at the 120-min (SMC = -0.85, 95%CI -1.32 to -0.38) and 180-min (SMC = -1.18, 95%CI -1.69 to -0.66) time points. A similar pattern was observed for lower-limb shear rate. No significant changes were observed for any outcomes in the upper limb. Subgroup analysis indicated that prolonged sitting decreased lower-limb FMD in healthy adults (SMC = -1.33, 95%CI -1.89 to -0.78) who had higher a priori vascular endothelial function, but not in those with metabolic and vascular dysfunction (SMC = -0.51, 95%CI -1.18 to 0.15). Interrupting sitting with active interruptions increased the standardised mean difference for FMD, relative to prolonged sitting, but it was not statistically significant(0.13, 95%CI -0.20 to 0.45).

Conclusion Lower-limb vascular function is progressively impaired as a consequence of prolonged sitting, up to 180 min. A similar trend was not observed in upper-limb vascular function. Subgroup analysis indicated prolonged sitting negatively impacts healthy populations, a finding not observed in those with metabolic disturbances. Regularly interrupting sitting with activity may be beneficial for those with metabolic disturbances.

Key words: Arteries; blood flow; sedentary behavior

INTRODUCTION

Excessive time spent in sedentary behaviours, defined as seated, reclined or lying posture with low energy expenditure ≤1.5 METS (1), is an independent risk factor for all-cause and cardiovascular disease (CVD)-related mortality (2, 3). Acute experimental studies have reported that prolonged periods of time spent sitting exacerbate postprandial cardiometabolic risk factors (4-6) and may result in transient vascular dysfunction (7-10). Impaired vascular function is an early and integral atherogenic event preceding morphological changes in the artery wall (11, 12). To date, two meta-analyses (8, 13) have summarised evidence on acute exposures to prolonged sitting, suggesting that sitting leads to a significant decline in vascular function in healthy adults. However, neither examined the time-course of vascular impairment and it is presently unknown whether there is a minimum amount of uninterrupted sitting which results in clinically relevant changes in vascular function and health.

Determining the time-course of vascular dysfunction is particularly important for clinical populations since impaired vascular reactivity represents a key stage in the pathogenesis of dysmetabolism (11). However, many acute experimental studies have focused on the effect of prolonged uninterrupted sitting in young and healthy populations (8), rather than older populations with metabolic disturbances. As a result, previous meta-analyses have only included healthy populations (8, 13) with only one meta-analysis reporting exposure to prolonged sitting leads to lower-limb vascular function (8). Given that those with metabolic disturbances spend a greater proportion of time in sedentary behaviour (14, 15), a greater understanding of the effects of prolonged uninterrupted sitting on vascular function in clinical populations is germane.

The World Health Organization has recently highlighted the need to quantify thresholds of prolonged sitting to determine the frequency and duration of activity interruptions (16). While studies indicate interrupting prolonged sitting can episodically improve vascular function (7, 17, 18), interruption strategies have reported mixed results. Likely due to the wide-range of methodologies employed when assessing flow-mediated dilation (FMD), the standard for vascular assessment (19). Other factors include different arterial sites and postures adopted during FMD assessments (8), and a lack of female participants (8, 10, 17, 20-26). Establishing the dose-relationship between vascular impairment and prolonged sitting across the dysmetabolism spectrum, and whether active interruptions in sitting are able to counteract sitting-related vascular impairment, is fundamental to informing larger trials and producing quantifiable sedentary behaviour public health recommendations.

The current systematic review, with meta-analysis, aimed to determine the dose-response relationship between acute prolonged uninterrupted sitting across multiple hours on upper and lower-limb vascular function, with additional sub-group analyses on the acute effect of prolonged sitting in (1) healthy adults relative to adults with metabolic disturbances, (2) older individuals relative to younger, (3) female participants compared to males, and (4) whether posture assessment influences vascular responses to prolonged sitting. A further aim was to compare prolonged uninterrupted sitting to episodic interventions involving brief activity interruptions.

METHODS

This systematic review with meta-analysis was reported in accordance with the Preferred

Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (PROSPERO trial registration number: CRD42020171394) (27).

Search strategy and study selection

Four electronic databases (Ovid Embase, Ovid Medline, PubMed, and CINAHL) were searched from inception to 23 June 2021 (4 December 2019, 23 September 2020 and 23 June 2021). The search strategy combined the terms in the following domains: exposure (sedentary OR "physical inactiv*" OR sitting OR "low physical activ*" OR "seated-rest" OR "bed-rest" OR "bed rest" OR lying OR supine OR inactiv*), outcome ("flow-mediated dilation" OR FMD OR "nitrate-mediated dilation" OR NMD OR "brachial artery ultrasound" OR "femoral artery ultrasound" OR "reactive hyper*" OR vasodilation OR "vascular function" OR "endothelial function"), and population (obes* OR "pre-diabet*" OR prediabet* OR "metabolic syndrome" OR metS OR diabet* OR T2D OR health* OR "cardio-metabolic" OR cardiometabolic OR dysmetaboli* or IGT or "impaired glucose tolerance" or IFG or "impaired fasting glucose" or overweight). The reference lists of all identified trials and relevant reviews were also examined.

Studies were imported into Endnote software (Clarivate Analytics, Philadelphia, USA), and duplicates were removed. Titles and abstracts of all identified records were screened, and relevant full-text articles were retrieved and reviewed by two independent reviewers (F.C.T. and A.J.P.). Discrepancies in inclusion or exclusion were resolved by consensus or through consultation with a third reviewer (D.W.D.).

Eligibility criteria

For the primary aim, manuscripts were eligible if they met the following criteria:

- Study design: randomised crossover trials and quasi-experimental.
- Population: adults and older adults (≥18 years) that represent key stages in the pathogenesis of type 2 diabetes (T2D): healthy, overweight (28), obese (28), impaired fasting glucose (29), impaired glucose tolerance (29), metabolic syndrome(30), and T2D (29).
- Exposure: prolonged, uninterrupted sedentary behaviour (1) period >30 min, but < 24 hours.
- Outcome: FMD was assessed pre- and post-prolonged sedentary behaviour and complied with standardised FMD protocol to ensure our protocol was comparable between studies (19). Whilst we acknowledge that current FMD guidelines typically stipulate assessments be performed in the supine position (19), movement between seating and supine would necessitate muscular activity that may impact FMD measures. For reasons of ecological validity in the context of the current analysis, studies of prolonged sitting that performed FMD measurements in the seated position were included.
- FMD time-points were 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged, uninterrupted sedentary behaviour. The following secondary outcomes were assessed pre- and after at 180 min of prolonged sedentary behaviour: blood flow, shear rate area under the curve (SRAUC), and mean arterial pressure (MAP).

For the secondary aim, studies were included if they additionally met the following criteria:

- Intervention: any light-intensity or moderate-to-vigorous physical activity that targeted interrupting sitting across multiple hours. To ensure that protocols were sufficiently homogeneous for comparison, only interventions with activity interruptions < 10 min in duration were included in meta-analyses.

Studies were excluded if participants consumed high-fat or high-carbohydrate meals before and/or during the trial.

Data extraction and quality assessment

The same reviewers carried out independent data extraction. Data were extracted relating to population characteristics (age, sex, body composition, and health status), exposure to sedentary behaviour (position and duration), FMD assessment (arterial site, method of collection), outcomes (FMD%, blood flow, SRAUC, and MAP), and details of any intervention aimed at interrupting sitting. Uncorrected FMD values were collected, as opposed to allometrically scaled values or those normalised to shear rate. If the data were unclear or were not available in the published manuscripts, the corresponding or first author was contacted by email to request this information.

Study quality was assessed using the Cochrane Risk of Bias Tool (31), which evaluates six domains of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. Each component was rated as “high risk” or “low risk”. If details for a particular domain were insufficient, the risk of bias was assessed as “unclear”. Two reviewers (F.C.T. and

A.J.P.) scored studies accordingly. In case of disagreements, a third reviewer (D.W.D.) evaluated the article.

Meta-analysis

All statistical analyses were performed using selected packages on R statistical software (version 3.6.1) (32, 33). Pre-to-post comparisons were calculated as the standardised mean change (MC) using pre- and post-prolonged sedentary behaviour data, pre-prolonged sedentary behaviour standard deviation (SD), sample size, and pre- to post-correlation. A random-effects model was used to determine the pooled effect estimate of all studies within the variable or subgroup as appropriate, with variance estimated through a restricted maximum likelihood (REML) model. The first analysis compared the effects of prolonged sedentary behaviour (30 min, 60 min, 120 min, 180 min, and ≥ 240 min) on FMD%, blood flow, SRAUC, and MAP response in upper and lower-limb arteries. For studies that had multiple time-points at ≥ 240 min, the time point closest to 240 minutes was used. A negative MC indicated that vascular function was impaired at that time point when compared to baseline (0 min). Subsequently, a sub-group analysis was performed at the ≥ 180 min time point to investigate the potential influence of (1) health status (healthy adults relative to adults with metabolic disturbances), and (2) posture in which vascular function was assessed. Meta-regressions were performed to examine the association between age and sex with SMC for lower-limb FMD at the ≥ 180 min time point. Sub-group analyses were only performed at ≥ 180 min as it contained the greatest number of studies (n = 16), and therefore the risk of reporting an exaggerated treatment effect was reduced (34). To assess the effects of interrupting prolonged sedentary behaviour, between-group comparisons (prolonged sedentary behaviour vs activity interruptions) were calculated as the effect size difference (SMD)

using pre- and post-intervention (FMD%), pre-intervention SD, sample size, and pre-to-post correlation for each group. Provided that none of the studies included in the meta-analysis presented pre- to post-correlation, this was estimated with data from our group (7). Where multiple arms involved different types of activity interruptions, we combined the sample size, mean and SD of both arms (35). Heterogeneity was measured using Higgin's I^2 test, and was interpreted by the following thresholds: 0–40%, might not be important; 30–60%, may represent moderate heterogeneity; 50–90%, may represent substantial heterogeneity; and 75–100%, considerable heterogeneity. A sensitivity analysis was carried out to identify the presence of highly influential studies by removing one study at a time, and then examining its effect on pre-to-post and between-group comparisons. Studies were considered as influential if removal resulted in a change of the SMC significance or magnitude. Publication bias was evaluated by visual inspection of the Begg's funnel plot when at least 8 trials were included in the meta-analysis. Significance was set at $p<0.05$ (two tailed). Data are presented as SMC or SMD and 95% confidence interval (CI).

RESULTS

Systematic Review

Study Inclusion

Figure 1 shows the PRISMA flow diagram. The systematic search resulted in the inclusion of 6,203 potential articles. Most were removed at abstract screening with 72 articles screened at full text. Most articles were excluded on the basis that they did not comply with standardised FMD protocol (n = 16; among them, 5 reported that the cuff was inflated for < 5 min, 5 reported that diameter was not continuously monitored, 5 reported that the post-deflation diameter was

monitored for < 3 min, and 1 did not report the complete FMD protocol) and did not include a control experimental condition (n = 9). Thirty-one studies were included in the systematic review.

Characteristics of Included Studies

The population and design of each study are reported in Supplementary Table 1 (see Table, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). From the 31 studies included in the analyses, vascular function was assessed in a total of 484 participants (healthy: 322 (9, 10, 17, 18, 20-26, 36-50); overweight or obesity: 121 (7, 51-53); metabolic syndrome: 5 (54); and T2D: 36 (42, 55). No participants from any of the included studies were described as having impaired fasting glucose or impaired glucose tolerance. Sample sizes were usually < 20 (median: 12 [range: 5 (54) to 56 (53)]), and most participants were male (n = 263/395 [67%]. One study did not report the sex of participants (53)). Typically, studies recruited young participants < 30 years (median: 26 years [range: 20.0 (10) to 61.5 (55)]), while populations with chronic disease risk factors or clinical conditions were likely to be older (median: 47.9 [range: 32.2 years (51) to 61.5 (55)]).

Bouts of prolonged sitting ranged from 0.5 h (44, 46) to 8.5 h (55), with the median duration of 3.0 h. The majority of study protocols assessed the impact of prolonged sitting on vascular function, however 3 studies utilised prolonged laying down (44, 47, 51) and 2 studies used undefined sedentary behaviour (40, 53). Assessments of FMD were mostly performed in the supine position (9, 10, 18, 20-23, 26, 37, 39, 41, 42, 44, 46-49, 51, 52), with only 8 studies performing(49) FMD in the seated position (7, 17, 24, 25, 38, 45, 54, 55) and 1 study in a semi-

recumbent position (36). Vascular function was predominantly assessed in the lower limb (n = 23; superficial femoral artery = 8 studies (7, 17, 18, 24, 25, 38, 46, 55); popliteal artery: 14 studies (9, 10, 20-23, 26, 36, 43, 45, 47-49, 52); posterior tibial artery: 1 study (41)), with only 12 trials assessing the brachial artery (7, 23, 25, 37, 39, 40, 42-44, 51, 53, 54). Assessments for FMD were recorded at 30-min (3 studies), 60-min (12 studies), 120-min (10 studies), 180-min (17 studies) and ≥ 240-min (10 studies).

Seven studies interrupted prolonged sitting via strategies including simple resistance activities (n = 2) (7, 55), walking (n = 3) (17, 18, 48), stair sprints (49) and calisthenics (n = 1) (37). Experimental protocols lasted between 1.4 h (37) to 8.5 h (49). Duration and frequency of interruptions in sitting ranged from 2 min of active interruption every 20 min of sitting (37) to 6 min every 60 min (55). From the 7 studies included in the analyses, vascular function was assessed in a total of 108 participants that were considered healthy (n = 65) (17, 18, 37, 48, 49), overweight or obese (n = 19) (7), or type 2 diabetic (n = 21) (55). Sample sizes were < 20, with the exception of Taylor *et al*. (2020) (55), and most included participants were male (n = 73).

Study quality

The quality score and risk of bias for each study are reported in Supplementary Figure 1 (see Figure, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Most studies included in the systematic review were randomised, with the exception of 7 studies that were quasi-experimental (22, 23, 25, 26, 36, 41, 45). All included studies were classified as low risk of bias for selective report. Only 5 studies provided details regarding allocation concealment (7, 21, 40, 48, 55). Most studies, with the exception of 2 (21, 40), did not report any form of blinding.

Only 10 out of the 31 studies reported blinding of the outcome assessor (7, 17, 21, 24, 25, 40, 45, 48, 51, 55). Most studies were classified as low risk of bias for incomplete outcome data. Finally, 1 study reported not controlling participants leg movement (18) and 1 study reported being underpowered to assess FMD% (37).

Meta-analysis

Study Inclusion

Of the 31 studies, 7 studies were excluded from the meta-analysis, as 5 did not impose prolonged sitting (40, 44, 47, 51, 53) and 2 reported duplicated data (17, 24). 24 studies were included in the meta-analysis to determine the dose-response relationship between acute prolonged uninterrupted sitting and upper and lower-limb vascular function. 7 studies were included in the meta-analysis to compare uninterrupted prolonged sitting to any acute physical activity intervention that targeted interrupting sitting.

Evaluation of the effects of prolonged sitting on vascular function

Prolonged sitting resulted in a significant decrease in lower-limb FMD at the 120-min (-0.85, 95% CI -1.32 to -0.38; see Supplementary Table 2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407) and 180-min (-1.18, 95% CI -1.69 to -0.66; Supplementary Table 2, http://links.lww.com/MSS/C407) time points. While no significant differences were observed at the 30-min, 60-min or ≥ 240-min time points ($p>0.31$ for all; Supplementary Table 2, http://links.lww.com/MSS/C407), there was a trend for lower-limb FMD to decrease as time spent sitting increased, from 30 min-to 180-min (see Supplementary Figure 2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Sensitivity analysis indicated that

none of the time-points unduly influenced the observed outcome, except for the 240-min time point, where removing a trial in healthy adults (48) significantly reduced lower-limb FMD (-0.40, 95% CI -0.92 to -0.12; Supplementary Table 2, http://links.lww.com/MSS/C407). Lower-limb shear rate was significantly reduced at 30-min (-0.52, 95% CI -0.87 to -0.16), 180-min (-0.77, 95% CI -1.01 to -0.54) and 240-min (-0.24, 95% CI -0.43 to -0.05) but no significant changes were observed at the 60-min or 120-min time point (see Supplementary Table 2; Supplementary Figure 3, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Sensitivity analysis at the 240-min time point indicated that removal of the least beneficial study (23), failed to elicit changes in shear rate. No significant changes were observed for lower-limb blood flow ($p>0.49$ for all), except for the 180-min time point (-1.00, 95% CI -1.61 to -0.39) (Supplementary Table 2, http://links.lww.com/MSS/C407). Sensitivity analysis indicated that none of the studies unduly influenced blood flow at any time points. MAP did not significantly change across any of the time points ($p>0.14$ for all). Sensitivity analysis indicated that none of studies unduly influenced MAP at any time points. For all upper-limb FMD measurements, no significant changes were observed at any of the time points ($p>0.10$ for all), with the largest pooled effect observed at the 240-min time point (-0.33, 95% CI -0.74 to 0.07; Supplementary Figure 2, http://links.lww.com/MSS/C407).

Subgroup analyses for FMD assessment were only performed on lower-limb FMD results at the $\geq$ 180-min time point. Subgroup analysis indicated that prolonged sitting resulted in a significant decrease in lower-limb FMD in healthy adults (-1.16, 95% CI -1.75 to -0.58), but not in adults with metabolic disturbances (-0.51, 95% CI -1.18 to 0.15; see Supplementary Figure 4, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Prolonged sitting

resulted in a significant decrease in lower-limb FMD irrespective of the position in which FMD was assessed (seated position: -1.25, 95% CI -2.23 to -0.26 and supine position: -0.82, 95% CI -1.27 to -0.37; see Supplementary Figure 5, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Meta-regression indicated a significant positive association between age and SMC for lower-limb FMD (β 0.04, 95% CI 0.01 to 0.07, p = 0.02; see Supplementary Figure 6, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). However, there was no influence of sex (β -0.01, 95% CI -0.02 to 0.01, p = 0.39; see Supplementary Figure 7, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407).

Evaluation of the effects of active interruptions in sitting on vascular function

Interrupting sitting with activity increased FMD, relative to prolonged uninterrupted sitting across multiple hours (0.13, 95% CI -0.02 to 0.45; Figure 3), but the result was non-significant. Sensitivity analysis indicated that none of the studies were influential to the analysis.. A non-significant increase was observed in both lower-limb FMD (0.12, 95% CI -0.33 to 0.56), and upper-limb FMD (0.13, 95% CI -0.38 to 0.65). Subgroup analysis for health status reported no change in FMD for healthy (0.00, 95% CI -0.49 to 0.49) and a non-significant increase for metabolic disturbances (0.29, 95% CI -0.12 to 0.69). Due to the low number of studies interrupting sitting, subgroup analysis for health status contained both upper- and lower-limb FMD results.

Publication bias and heterogeneity

Visual inspection of the funnel plot at ≥ 240-min revealed no asymmetry (see Supplementary

Figure 9, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407), however 180-min revealed some asymmetry (see Supplementary Figure 8, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). However, trim and fill analysis showed that imputing missing studies to reduce asymmetry did not significantly change the effect size. The heterogeneity was moderate to substantial for time-points 60-min to 240-min which may be partially explained by varying methodology (arterial sites measured, posture transitions) and population groups (healthy, metabolic syndrome, T2D). For lower limb shear rate, there was considerable heterogeneity at the 60-min and 120-min mark, which may reflect the small number of studies reporting shear rate (Supplementary Table 2, http://links.lww.com/MSS/C407). For lower limb blood flow, heterogeneity was considerable for all time points, which again may reflect the small number of studies reporting blood flow. Visual inspection of the funnel plots at the 180-min for shear rate, blood flow and MAP did not reveal any substantial asymmetry.

DISCUSSION

The aim of this systematic review was to determine the dose-response relationship of prolonged uninterrupted sitting (> 30 min) with upper- and lower-limb vascular function across multiple hours during the day. Prolonged uninterrupted sitting for 120 and 180 min significantly decreased lower-limb vascular function, but did not impact upon upper limb function. Whilst no statistically significant decrease was observed at the 30-min, 60-min, there was a clear trend for lower-limb vascular function to decline as time spent sitting increased. A similar pattern was observed for lower-limb shear rate. Subgroup analysis indicated that prolonged sitting was detrimental to young healthy individuals, with this negative effect being less pronounced in adults with metabolic disturbances and older adults. Finally, interrupting sitting with activity

resulted in a small non-significant increase in FMD, relative to prolonged sitting, for those with metabolic disturbances.

Time-course to vascular impairment

This is the first meta-analysis to assess the time-course of impairment in vascular function. A major implication is these findings suggest that 120-min of continuous prolonged sitting may represent a critical threshold for lower-limb vascular susceptibility. There are several reasons why this decline in lower-limb vascular function may be observed. Notably, a corresponding decrease in shear rate was observed. Shear stress is the frictional force exerted on the arterial wall (56) and it is recognised as the key physiological stimulus in maintaining endothelial health (57). The progressive reduction in lower-limb shear stress from 30 min to 180 min may be partly responsible for the corresponding decline in FMD%. It was recently suggested that changing shear patterns may be responsible for the reduced FMD% following prolonged sitting (8). While our meta-analysis did not address changes in the patterns of shear, the absence of antegrade shear in the presence of increased retrograde shear has been shown to decrease FMD responses (56, 58, 59). Reduced muscle activity and increased pressure in the back of the thighs are also likely contributors (60). Indeed, lower-limb shear rate decreased at all timepoints relative to pre-sitting in the same stepwise manner as FMD%.

In contrast to our lower limb findings, there were no significant changes in upper-limb FMD responses at any time points. Meta-analyses have observed similar findings in healthy populations following 180-mins of sitting (8). Maintenance of shear stress by continuation of desk-based activities whilst sitting has been postulated to contribute to the preservation of

brachial artery FMD (8). Indeed, a lack of upper-limb shear stress reduction relative to lower-limb FMD was observed at all timepoints. Whilst studies examining the vascular effect of upper-limb inactivity during sitting are needed to clarify this hypothesis, the exercise training literature may provide additional insights. In response to lower-limb cycle training, significant vascular functional changes have been observed in untrained upper limbs, likely due to the relatively large systemic effects of large muscle group exercise on systemic hemodynamics and shear stress (61, 62). The lack of change in upper-limb vascular function following prolonged sitting may reflect the relatively modest impacts on shear rate associated with sitting. Reduced lower-limb shear may impact brachial artery vascular function over days, weeks, months and years. Indeed, bed rest studies assessing vascular function over multiple weeks have demonstrated sedentary behaviour to be a strong stimulus for rapid structural remodelling of resistance and conduit arteries. Future studies that measure vascular function more frequently, across weeks and months, are needed to provide greater insight into the time-course of vascular change and adaptation in response to prolonged sitting in both the upper- and lower-limbs.

Subgroup Analyses

For timepoints 60 – 180 min (Figure 2), a reduction of 1% or greater was observed in FMD. A 1% chronic reduction in FMD has been associated with a 13% increase in future risk of cardiovascular events (12, 63). Although the data in this study is reflective of acute changes, repeated exposures to This is additionally concerning for high-risk and clinical populations, where reduced vascular function can further compound pre-existing cardiovascular risk factors such as older age, obesity, and T2D (64). Further, older individuals and those with metabolic disturbances typically spend more time in sedentary behaviour relative to young and healthy

individuals (15, 65-68), further contributing to their CVD risk. Despite this, most participants within this meta-analysis were young and healthy. Given that preserving or improving vascular function in these populations are fundamental to reducing atherosclerotic development, understanding the impact of prolonged uninterrupted sitting is imperative to quantify prolonged sitting thresholds and inform public health recommendations.

We observed that prolonged sitting had less apparent impact on vascular function in older adults and those with metabolic disturbances than in young healthy subjects. This may reflect the law of initial values (69). Given that older and diseased populations possess impaired vascular function *a priori*, the magnitude of response to prolonged sitting appears reduced. However, the small sample of older adults and those with metabolic disturbances makes it difficult to draw conclusions as to how prolonged uninterrupted sitting may influence individuals across the full spectrum of age and metabolic function. There also continues to be a primary focus on male participants in this research space. Eleven of 31 studies recruited only male participants (10, 17, 21-26, 38, 49, 54), and an additional 13 studies recruited majority male participants (7, 9, 18, 20, 37, 41, 42, 44, 46-48, 52, 55). Given many studies have reported excessive sitting time in female populations from young, healthy females (70) to older females experiencing cardiovascular complications (71), it is necessary to increase female participant recruitment in the area of vascular function and sedentary behaviour. It is conceivable that sex differences may be present in the impact of prolonged sitting, given the well described hormonal impacts on endothelial function (72). Continued failure to recruit females, older adults and individuals with pre-existing cardiovascular risk factors not only widens the existing gap in the literature but makes it difficult to provide public health recommendations for a large portion of the population.

Prolonged Uninterrupted Sitting and Vascular Assessment

An area of contention in previous studies of prolonged inactivity is the posture adopted during FMD assessment (8). While many studies assumed the supine position during assessment, 8 of the studies measured vascular function whilst sitting. Sub-analysis of seated versus supine assessment in the lower-limb demonstrated that regardless of posture, vascular function significantly decreased when prolonged uninterrupted sitting was 180 min. Whilst there was considerable heterogeneity in the seated position, there were also fewer studies (n = 8) and a greater variability in participant health and age. When designing protocols that utilise FMD for vascular assessment, posture should be taken into consideration. If movement between seated and supine is active, increases in muscular activity may influence FMD results (25, 55). Future research is needed to assess the validity and reliability of seated FMD measures.

Interrupting Sitting with Activity

Despite growing evidence indicating reducing and interrupting prolonged sitting time positively influences glucose metabolism and shear stress profiles across the metabolic risk spectrum (73, 74), limited research exists investigating the effects of regularly interrupting sitting on vascular function (8). Our meta-analysis included 7 trials, and demonstrated a non-significant increased FMD response (0.13, 95%CI -0.20 to 0.45) when sitting was regularly interrupted with activity compared to prolonged, uninterrupted sitting over a few hours. Responses were similar between upper and lower-limb vascular function relative to upper limb for interrupting sitting, which may be attributable to protocol design. Protocols assessing lower-limb vascular function were typically longer in duration and thus participants were exposed to more frequent sedentary bouts. It is possible that a greater level of activity (increased frequency and duration) is needed to

correspond to increased time spent in sedentary behaviour.

Recent meta-analyses have demonstrated similar improvements in both short- and long-term sedentary behaviour interventions (8, 75). However, the varying interruption strategies including mode, duration and frequency make it difficult to identify optimum activity interruptions. Nevertheless, this literature indicates that those with metabolic disturbances benefit from an increase in FMD following activity interruptions.

Limitations

This study was the first meta-analysis to examine the time-course between prolonged sitting and vascular function in both upper- and lower-limbs. Moreover, we provide additional recommendations for future research based on existing gaps in literature including health status, age and sex. However, there are limitations to address. It is important to acknowledge there are a small number of studies at the 30 min and 60 min time points. Therefore, it is possible that with additional data, vascular impairment may be revealed earlier than 120 min. Given decreased vascular function has been reported as early as 30-mins (7), future research should consider measuring vascular function at earlier time points. Secondly, our search criteria did not include terms surrounding 'activity interruptions'. However, given that to be eligible for study selection the studies needed to compare to prolonged sitting we feel that we captured these articles in our search. Finally, as previously addressed, there are several gaps in the research with regard to female participants, and older and clinical populations.

CONCLUSION

Prolonged uninterrupted sitting progressively impairs lower-limb vascular function up to 180 min. In healthy populations vascular function may be more susceptible to the impacts of prolonged sitting given those with metabolic disturbances report reduced vascular function *a priori*. Regularly interrupting sitting may improve vascular function for with metabolic disturbances, however more data is needed to clarify. Future studies should also aim to include a wider range of participants including females, older adults and adults further along the metabolic spectrum to address to large gap in the literature. The findings of our analysis strongly suggest that prolonged uninterrupted sitting is detrimental for arterial function and health and that recommendations should focus on interrupting sitting with regular activity breaks spaced at most 120 mins apart.

GRANTS

This research was not supported by any grants.

DISCLOSURE

No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. All authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

PROSPERO trial registration number CRD42020171394.

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FIGURE LEGEND

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of the literature search results.

Figure 2. Effects of active interruptions on vascular function (FMD) meta-analysis using a random-effects model grouped by upper- and lower-limb assessment. *SMD* standardised mean difference, *CI* confidence intervals.

Figure 3. Mean time-course change for raw FMD% after 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged uninterrupted sitting. A) changes in FMD% in the upper limb, B) depicts changes in FMD% in the lower limb. Black square and error bars are presented as mean change (95% CI). Dots represent the mean change for each individual study. Green dots, healthy adults; red dots, adults with metabolic disturbances. Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb FMD data reported at the 30 min.

SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content1. docx. APPENDIX

Figure 1

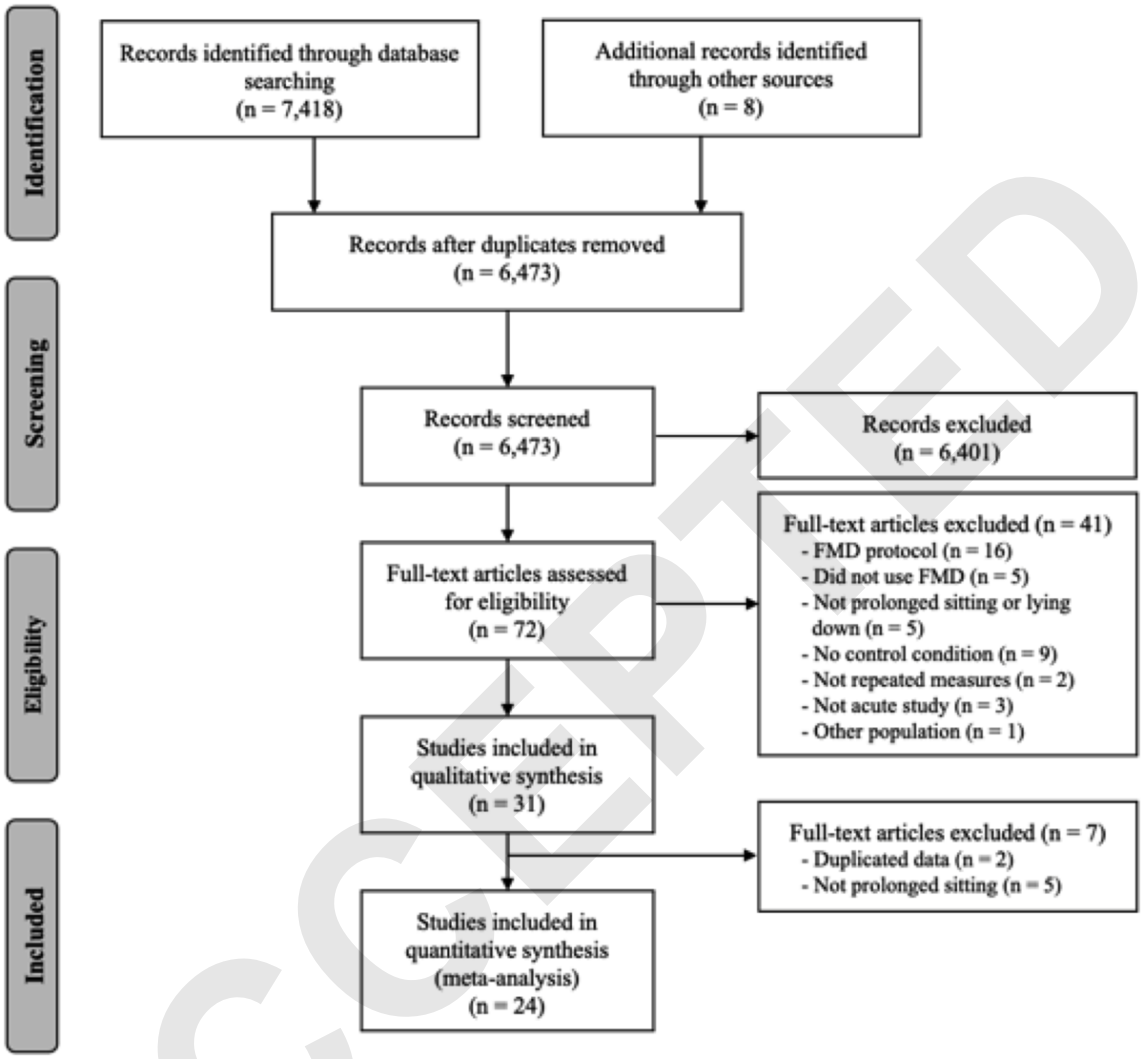


Figure 2

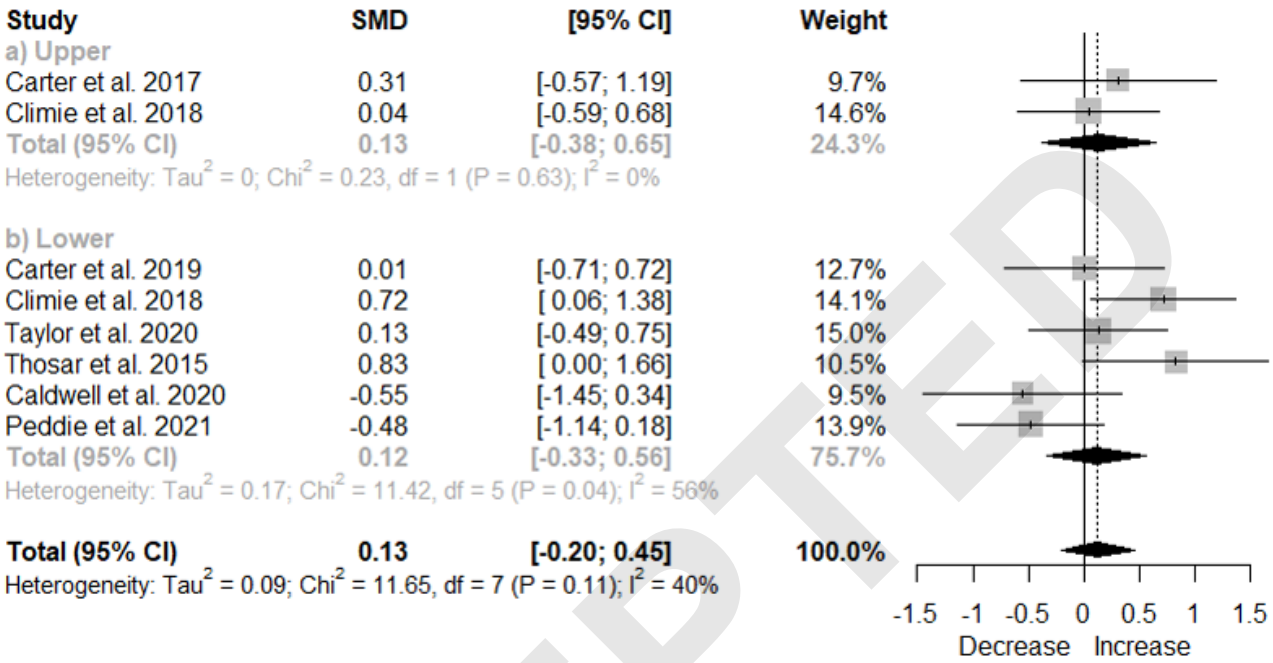
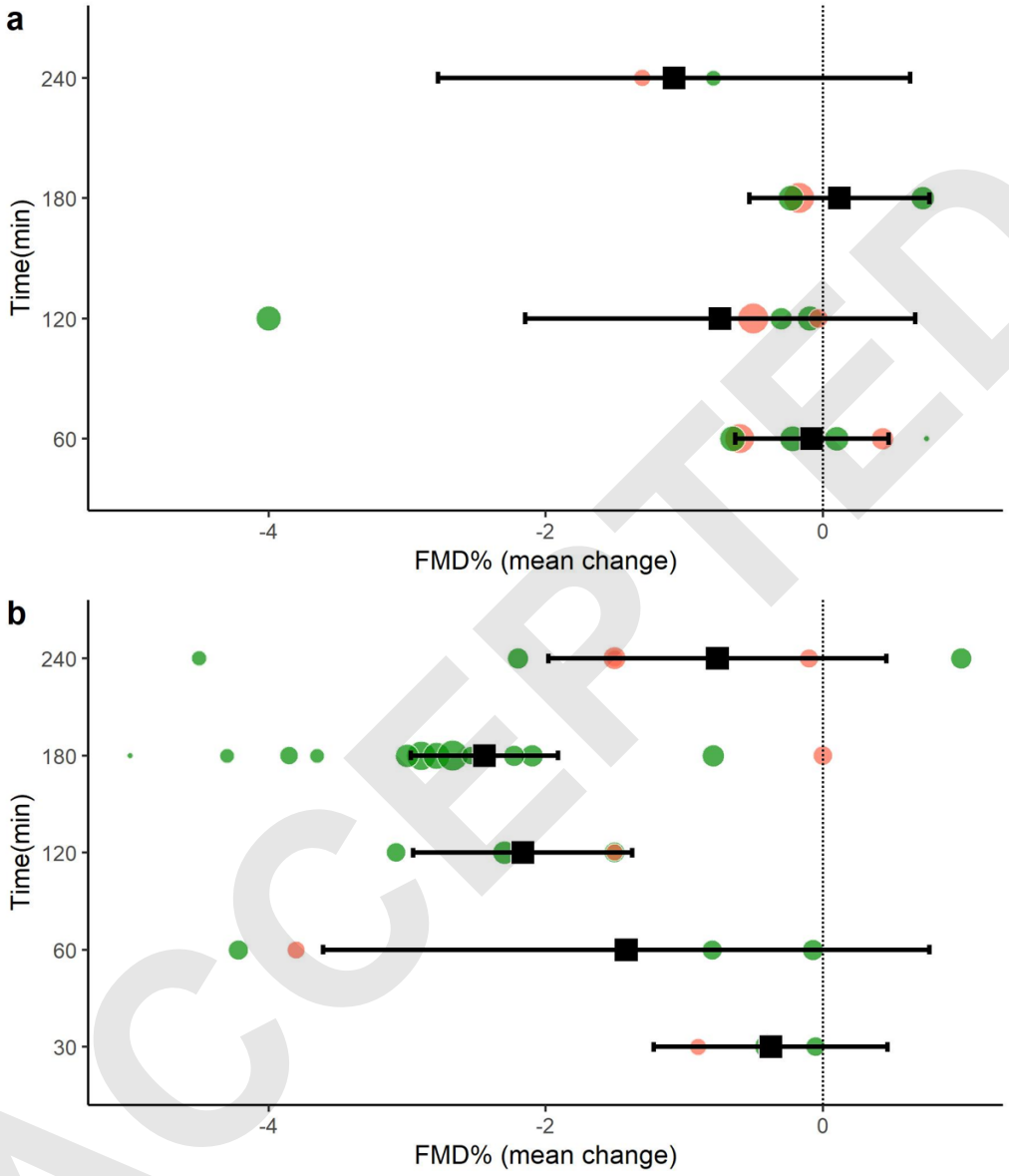


Figure 3



Supplementary Material

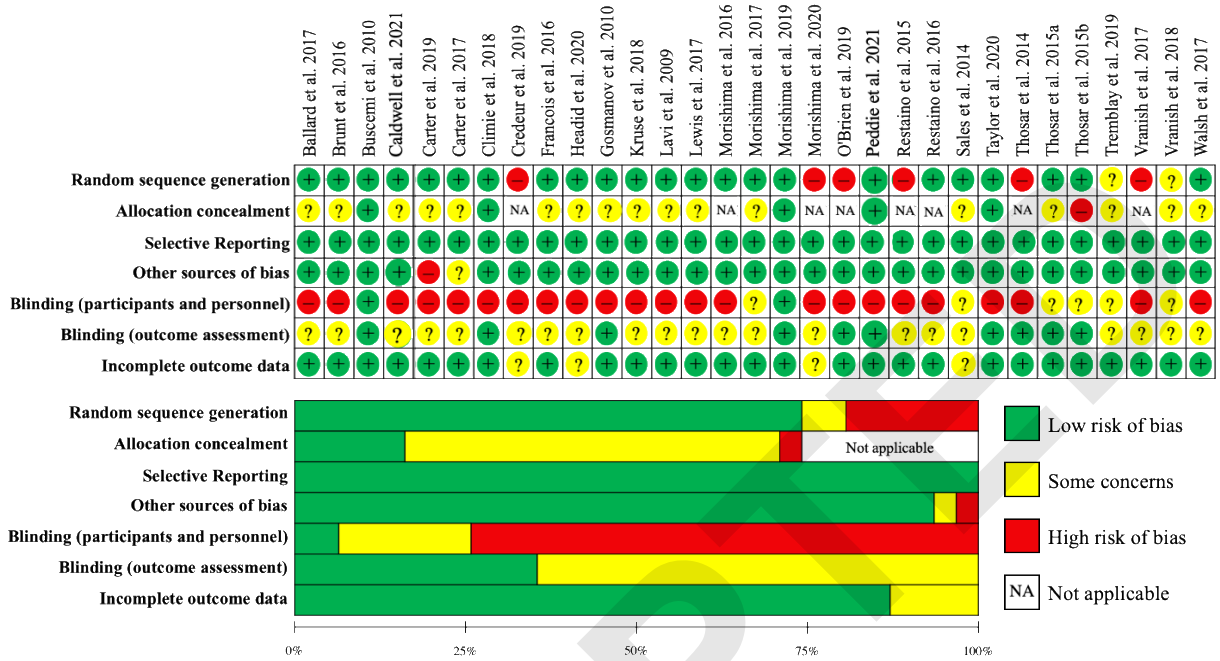


Figure 1. Cochrane Collaboration's risk of bias (RoB) for the included studies. Green, low risk of bias; Red, high risk of bias; Yellow, unclear risk of bias.

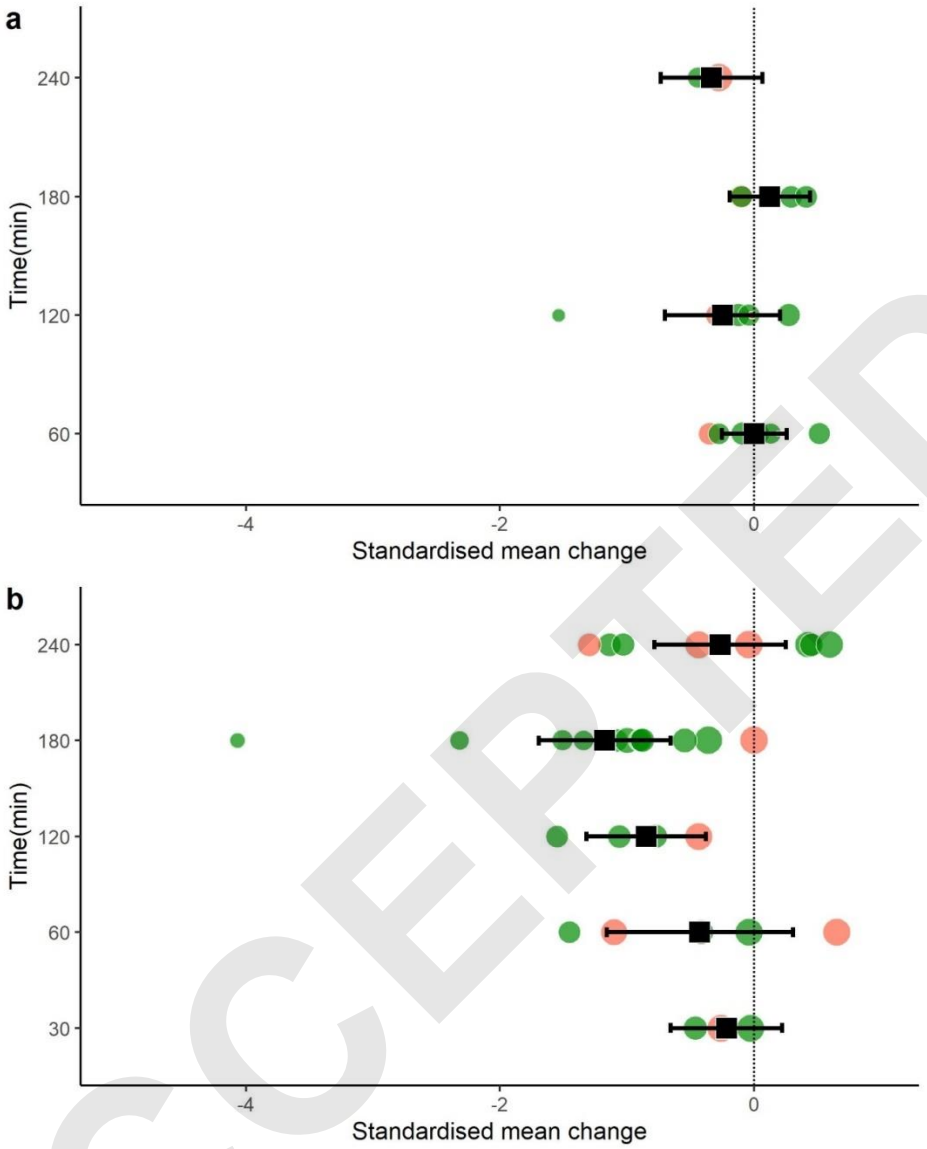


Figure 2. Time-course for standardised mean change in FMD after 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged uninterrupted sitting. A) changes in SMC in the upper limb, B) depicts changes in SMC in the lower limb. Black square and error bars are presented as standardised mean change (95% CI). Dots represent the standardised mean change for each individual study. Green dots, healthy adults; red dots, adults with metabolic disturbances. Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb FMD data reported at the 30 min.

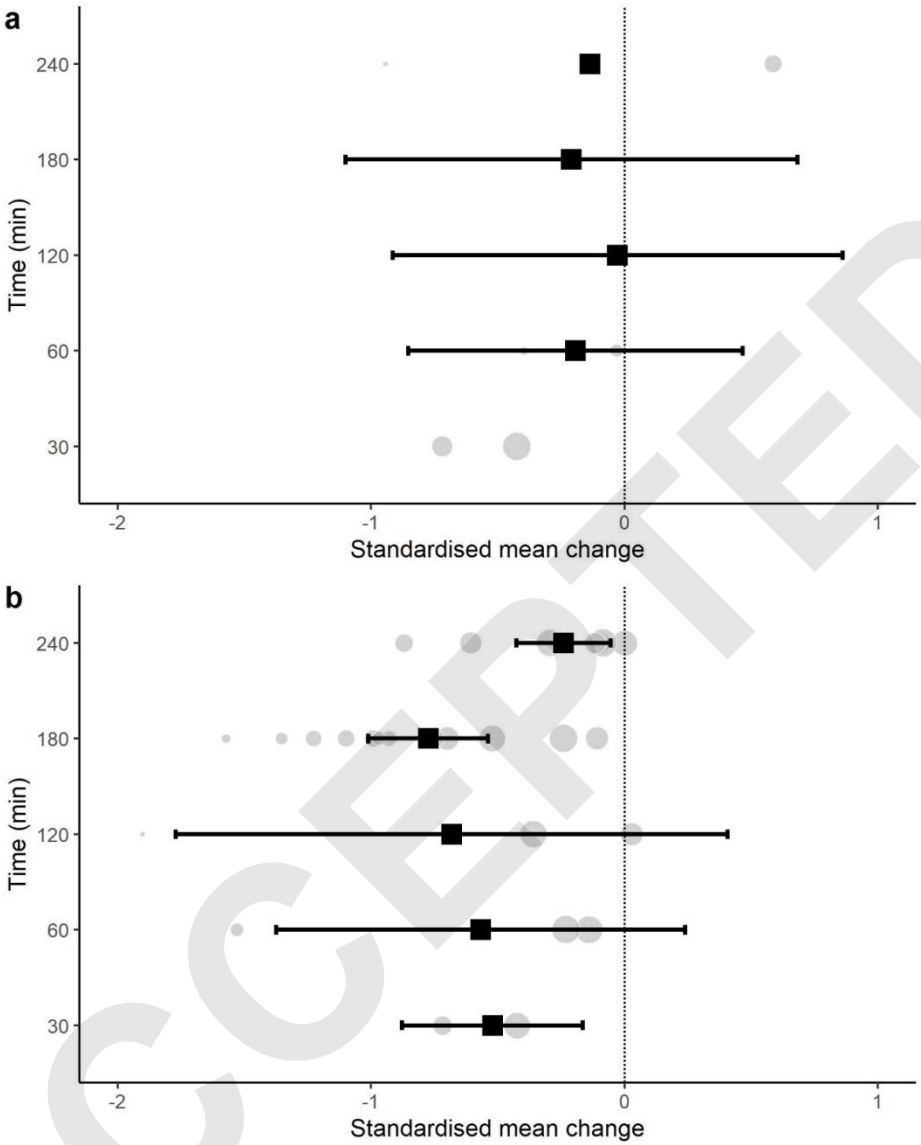


Figure 3. Time-course for standardised mean change in SRAUC after 30 min, 60 min, 120 min, 180 min, and ≥ 240 min of prolonged uninterrupted sitting. A) changes in SMC in the upper limb, B) changes in SMC in the lower limb. Data are presented as standardised mean change (95% CI). Dots sizes are proportional to the weight of each study in the analysis. There was no upper-limb SRAUC data reported at 30 min.

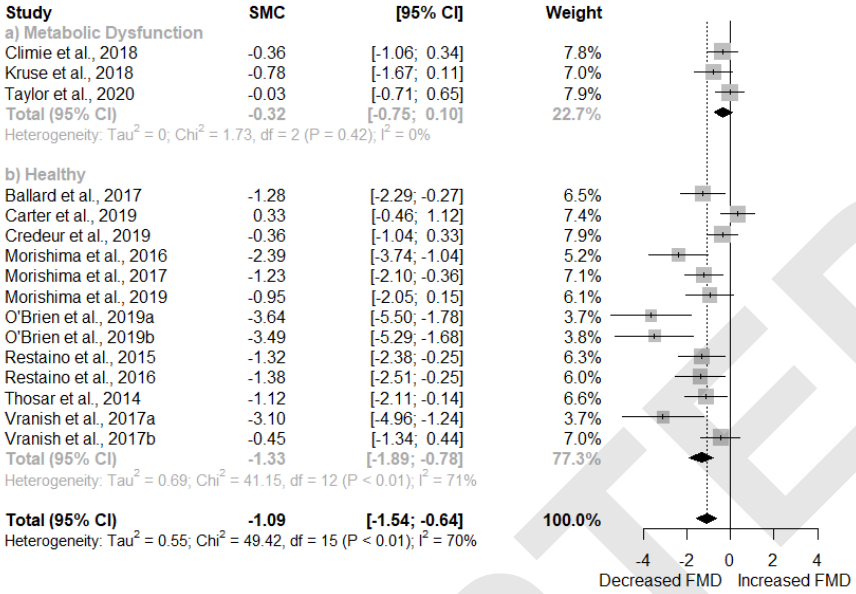


Figure 4. Effect of ≥180 min prolonged sitting on lower-limb vascular function (FMD) meta-analysis using a random-effects model grouped by health status. *SMC* standardised mean change, *CI* confidence intervals.

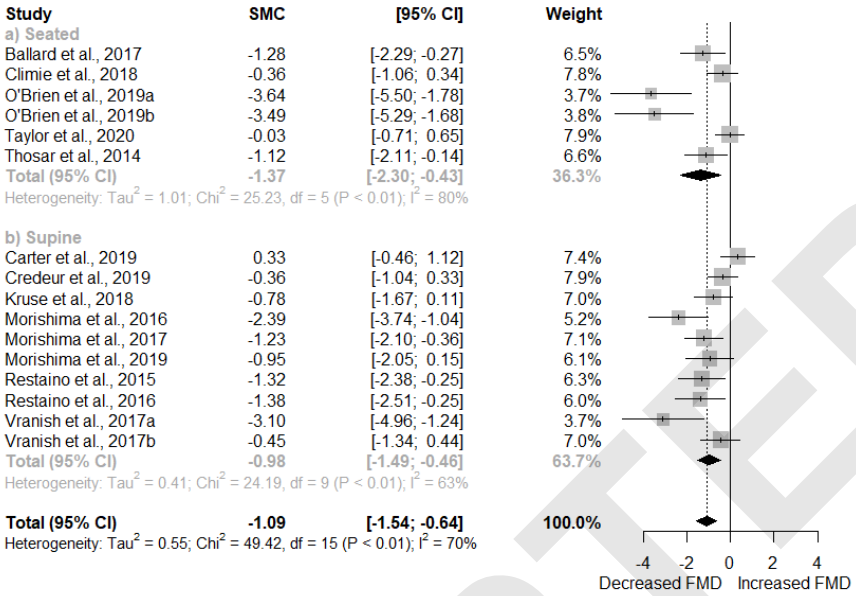


Figure 5. Effect of ≥ 180 min prolonged sitting on lower-limb vascular function (FMD) meta-analysis using a random-effects model grouped by posture during FMD assessment. *SMC* standardised mean change, *CI* confidence intervals.

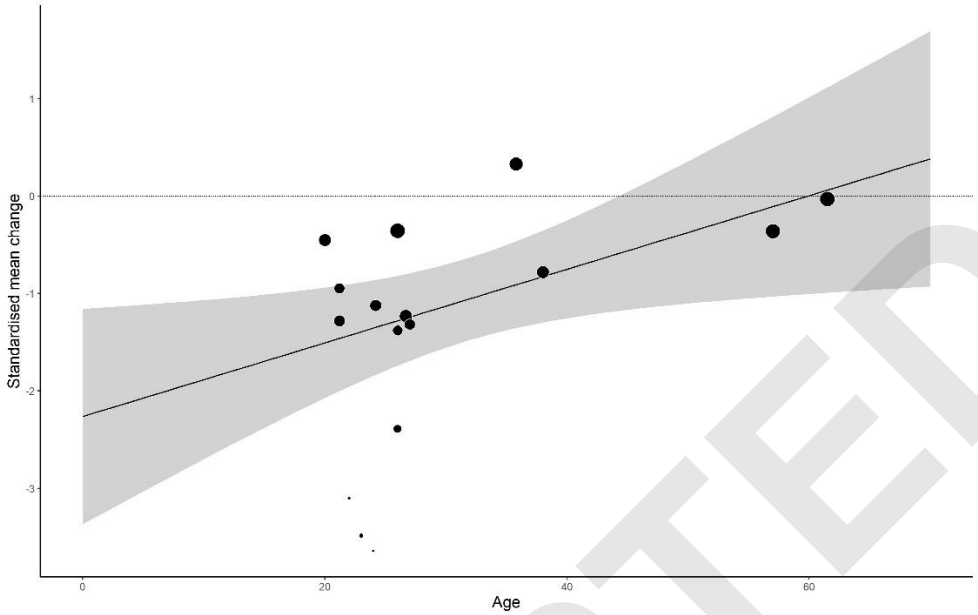


Figure 6. Meta-analytic bubble plot of age of participants against FMD. Dots sizes are proportional to the weight of each study in the analysis.

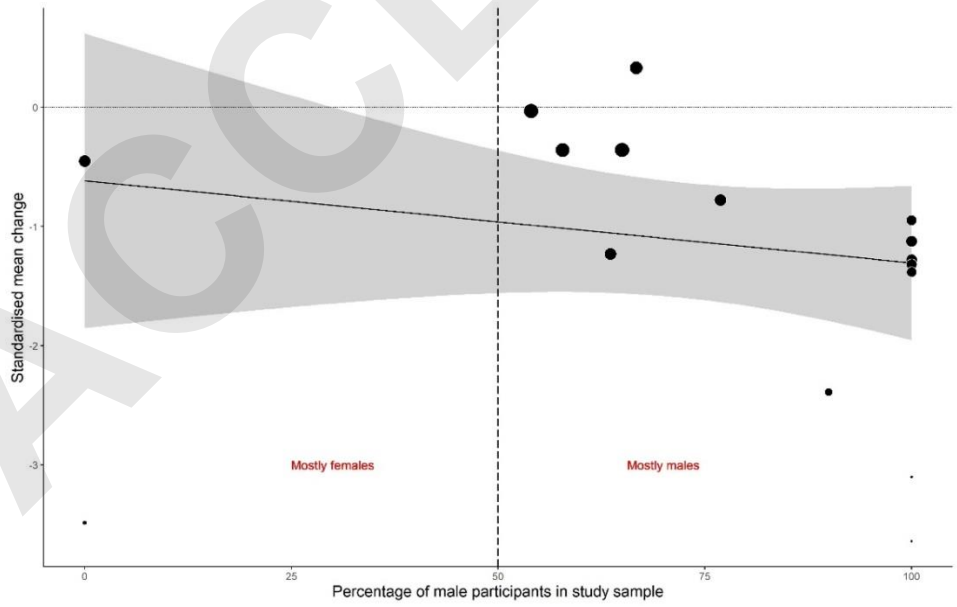


Figure 7. Meta-analytic bubble plot of percentage of male participants against FMD. Dots sizes are proportional to the weight of each study in the analysis. Mostly females, studies with more

than 50% female participants; mostly males, studies with more than 50% male participants.

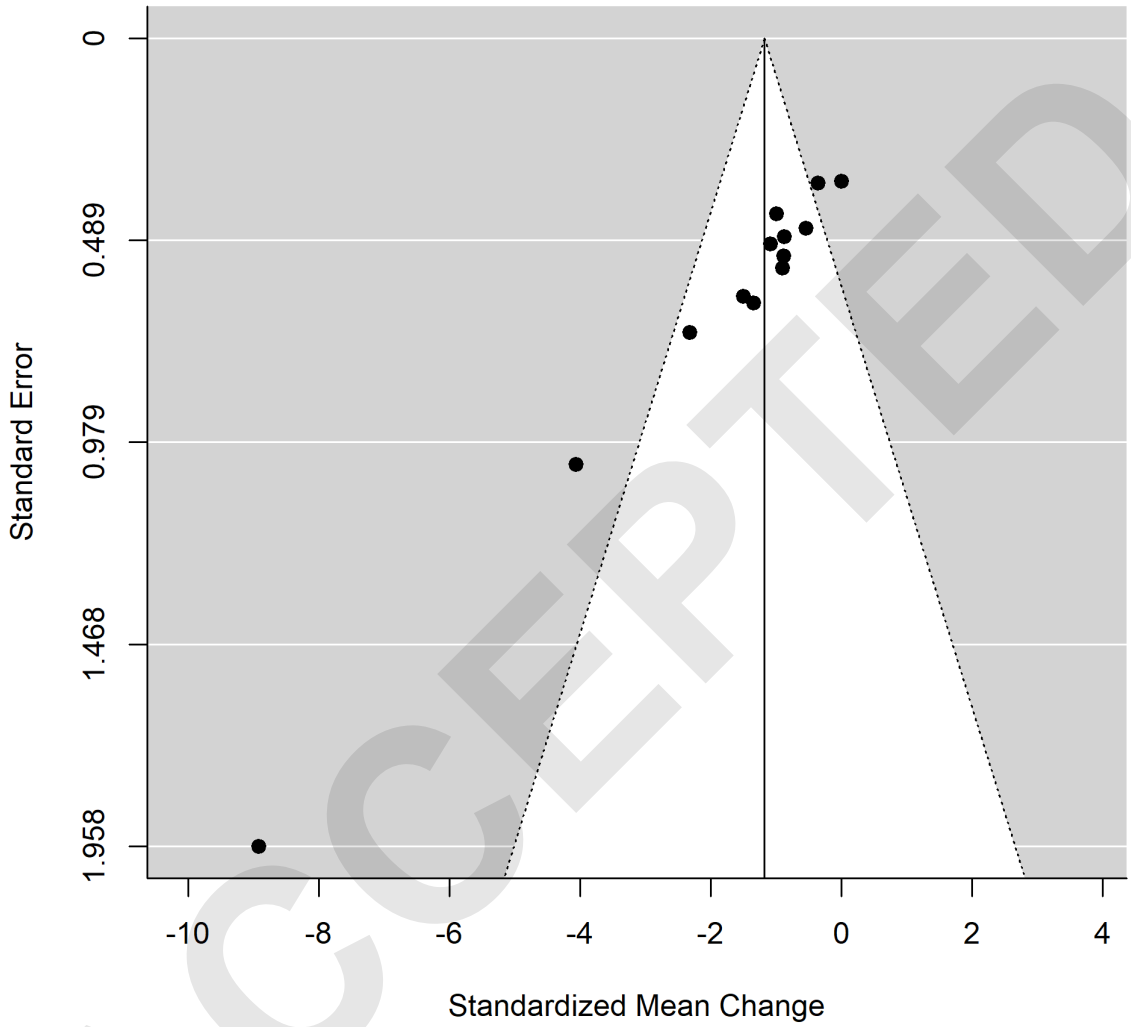


Figure 8. Funnel plot for effect of 180 min prolonged uninterrupted sitting on lower-limb vascular function meta-analysis.

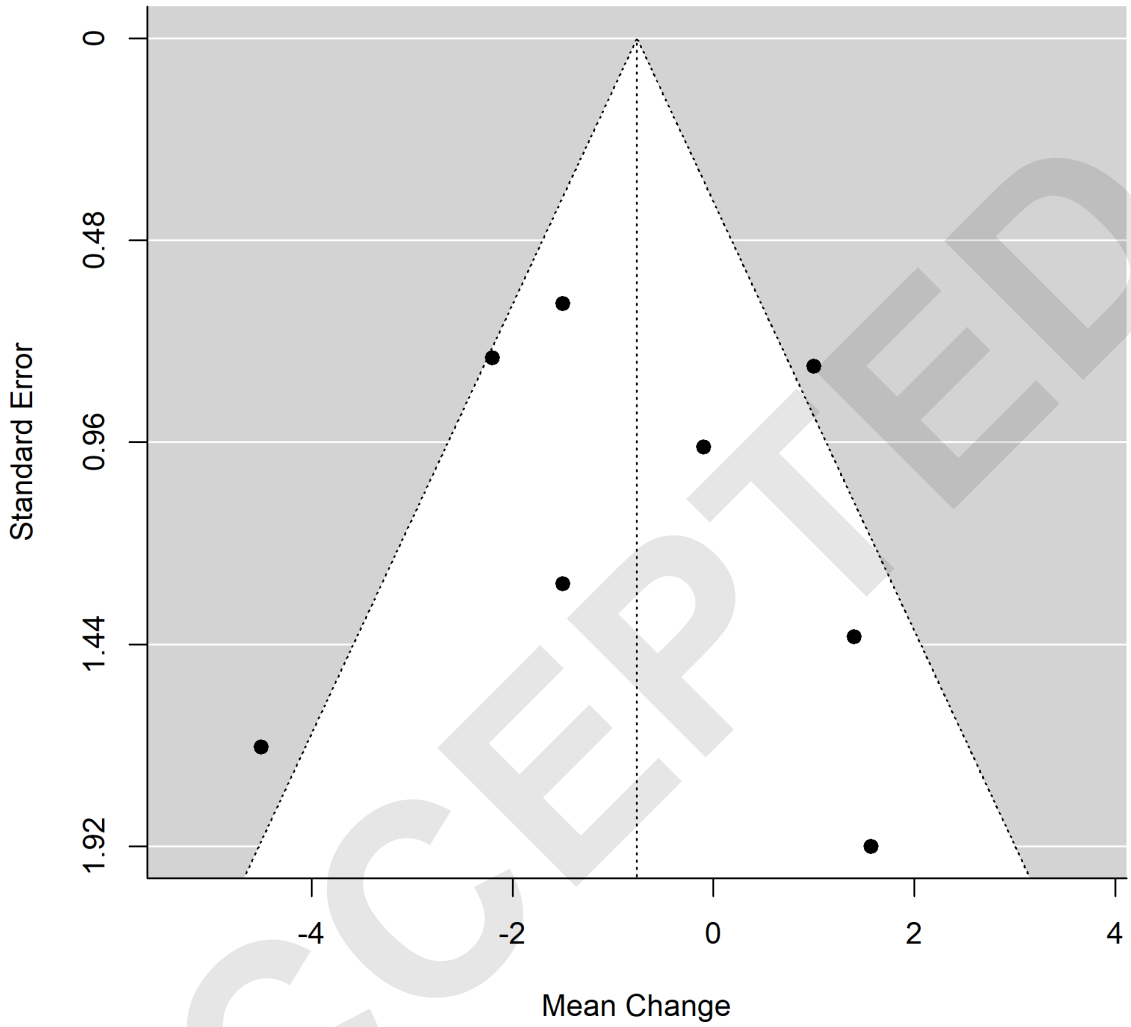


Figure 9. Funnel plot for effect of ≥240 min prolonged uninterrupted sitting on lower-limb vascular function meta-analysis.

TABLES

Table 1. Characteristics of the included trials.

Author	Population	Study design	Protocol	Time – points (minutes)	Outcomes
Ballard et al. 2017 [38]	12 M; age: 21.2 ± 1.9 yr; BMI: 24.6 ± 1.1 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 135 + 180 min Meal: 58/14/6 g, 382 kcal	-135, 0, 60, 120, and 180	FMD - seated femoral artery, base diameter, SRAUC, and MAP
Brunt 2016 [39]	5 M 5 F; age: 23 ± 6 yr BMI: 22.8 ± 1.7 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 100 min Meal: none	0 and 100	FMD - supine brachial artery, base diameter, and SRAUC
Buscemi et al. 2010 [40]*	10 M 10 F; age: 31 ± 2 yr BMI: 23.9 ± 0.7 kg/m² healthy	RCT double-blinded	Prolonged SB (placebo condition) Duration: 60 min Meal: decaffeinated coffee	0, 30, and 60	FMD - brachial artery
Caldwell et al. 2021 [49]	10 M; age: age: 24 ± 4 yr BMI: 24 ± 2 kg/m² healthy	RCT	Prolonged sitting Duration: 540 min	-30 and 510	FMD – femoral artery and SRAUC
Carter et al. 2017 [37]	6 M 4 W; age: 27.3 ± 8.3 yr healthy, active	RCT	Prolonged sitting Duration: 86 min Meal: none	0 and 86	FMD - supine brachial artery and MAP

Carter et al. 2019 [18]	10 M 5 F; age: 35.8 ± 10.2 yr BMI: 25.5 ± 3.2 kg/m² healthy, active	RCT	Prolonged sitting Duration: 240 min Meal: 61/8/5 g, 320 kcal	0 and 240	FMD - supine femoral artery, base diameter, blood flow, SRAUC, and MAP
Climie et al. 2018 [7]	11 M 8 F; age: 57 ± 12 BMI: 30.6 ± 3.4 overweight and obese, sedentary and inactive	RCT	Prolonged sitting Duration: 300 min Meal: 53-55%/30-33%/12-15%	Brachial: 0, 30, 60, 120, and 300 Femoral: 0 and 300	FMD - seated femoral and brachial artery, base diameter, blood flow, and SRAUC
Credeur et al. 2019 [41]	13 M 7 F; age: 26 ± 7 yr BMI: 30 ± 7 kg/m² healthy, overweight, and obese 6 M 6 F; age: 57.5 ± 5.0 yr BMI: 35 ± 7 kg/m² type 2 diabetes	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 80	FMD - supine posterior tibial artery, base diameter, SRAUC, and MAP
Francois et al. 2016 [42]	6 M 6 F; age: 55.3 ± 9.1 yr BMI: 26 ± 5 kg/m² healthy, inactive 7 M 4 F; age: 55.1 ± 7.0 yr BMI: 23 ± 3 healthy, active	RCT	Prolonged sitting Duration 140 min Meal: none	0, 60, 120 and 180	FMD - supine brachial artery, base diameter, blood flow, and MAP
Gosmanov et al. 2010	4 M 9 F; age: 32.2 ± 9.8 yr	RCT	Prolonged lying down	0, 240 and 480	FMD - supine

[51]	BMI: 36.7 ± 5.1 kg/m² obese		(placebo condition) Duration: 480 min Meal: none		brachial artery
Headid et al 2020 [43]	6 M 6 F; age: 22.3 ± 2.0 yr BMI: 23.9 ± 3.0 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 150 min Meal: none	0 and 150	FMD - brachial and popliteal artery and MAP
Kruse et al. 2018 [52]	10 M 3 F; age: 38 ± 3 yr BMI: 29.7 ± 2.0 kg/m² overweight and obese, inactive	RCT	Prolonged sitting Duration: 240 min Meal: 46/9/16 g, 310 kcal	0 and 240	FMD - supine popliteal artery, base diameter, blood flow, and SRAUC
Lavi et al. 2009 [53]	56 participants; age: 47.9 ± 5.8 yr BMI: 32.1 ± 4.3 kg/m² overweight and obese	RCT	Prolonged SB (placebo condition) Duration: 120 min Meal: none	0 and 120	FMD - brachial artery
Lewis et al. 2017 [44]	9 M 1 F; age: 27 ± 2 yr BMI: 23 ± 2 kg/m² healthy	RCT	Prolonged lying down Duration: 30 min Meal: none	0 and 30	FMD - supine brachial artery, base diameter, blood flow, and SRAUC
Morishima et al. 2016 [9]	7 M 4 F; age: 26 ± 1 yr BMI: 25.0 ± 1.1 kg/m² healthy, recreationally active	Unilateral model	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP

Morishima et al. 2017 [20]*	10 M 6 F; age: 26.7 ± 0.5 yr BMI: 25.6 ± 0.5 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 45 + 180 min Meal: none	-45 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Morishima et al. 2019 [21]*	9 M; age, 21.2 ± 2.0 yr BMI, 22.0 ± 3.0 kg/m² healthy, recreationally active	RCT	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Morishima et al. 2020 [22]	9 M; age, 21.1 ± 1.8 yr BMI 24.8 ± 1.5 kg/m² healthy, recreationally active	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
O'Brien et al. 2019 [45]	10 M; age, 24 ± 2 yr BMI, 26.6 ± 2.0 kg/m² 10 F; age, 23 ± 2 y BMI, 24.2 ± 3.2 kg/m^2 healthy, active	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - seated popliteal artery, base diameter, blood flow, SRAUC, and MAP
Peddie et al. 2021 [48]	11 M 7 F; age, 23.5 ± 5 yr BMI: 23.7 ± 2.6 kg/m² healthy, sedentary (≥ 5 h/day of sitting)	RCT	Prolonged sitting Duration: 360 min Meal: 62%/28%/10%	-30 and 360 min	FMD – supine popliteal artery, base diameter, and blood flow
Restaino et al. 2015 [23]	11 M; age, 27 ± 1 yr	Quasi-experimental	Prolonged sitting	0 and 180	FMD - supine brachial and

	BMI: 25 ± 0.4 kg/m² healthy, recreationally active		Duration: 180 min Meal: none		popliteal artery, base diameter, blood flow, and SRAUC
Restaino et al. 2016 [10]*	10 M; age, 26 ± 1 yr BMI, 26.8 ± 1.3 kg/m² healthy, recreationally active	Unilateral model	Prolonged sitting Duration: 180 min Meal: none	0 and 180	FMD - supine popliteal artery, base diameter, blood flow, SRAUC, and MAP
Sales et al. 2014 [54]*	5 M; age, 39 ± 3 yr BMI, 31.4 ± 0.8 kg/m² MetS, sedentary	RCT	Prolonged sitting Duration 60 min + 60 min Meal: none	-60, 30 and 60	FMD - seated brachial artery and base diameter
Taylor et al. 2020 [55]	13 M 11 F; age: 61.5 ± 7.8 yr; BMI: 32.6 ± 3.5 kg/m² T2D with overweight or obesity, sedentary	RCT	Prolonged sitting Duration: 420 min + 60 min; Meal: 53-55%/30-33%/12-15% breakfast and lunch	0, 60, 210, 270 and 420	FMD - seated femoral artery, base diameter, blood flow, and SRAUC
Thosar et al. 2014 [25]	12 M; age, 24.2 ± 4 yr BMI, 23.7 ± 3.3 kg/m² healthy, inactive	Quasi-experimental	Prolonged sitting Duration: 180 min Meal: none	0, 60, 120 and 180	FMD - seated femoral and brachial artery, base diameter, and SRAUC
Thosar et al. 2015a [17]	12 M; age, 24.2 ± 4 yr BMI, 23.7 ± 3.4 kg/m² healthy, inactive	RCT	Prolonged sitting Duration: 180 min Meal: none	0, 60, 120 and 180	FMD - seated femoral artery
Thosar et al. 2015b [24]	11 M; age, 24.2 ± 4.4 yr	RCT	Prolonged sitting	0, 60, 120 and 180	FMD - seated femoral artery

	BMI: 23.6 ± 3.4 kg/m²		Duration: 180 min		
	healthy, inactive		Meal: none		
	9 M 2 F; age: 23 ± 2 yr		Prolonged sitting		FMD - supine femoral artery, base diameter, SRAUC, and MAP
Tremblay et al. 2019 [46]	BMI: 24 ± 3 kg/m²	RCT	Duration: 30 min	0 and 30	
	healthy		Meal: none		
	8 M; age: 22 ± 1 yr				
	BMI: 25.7 ± 0.9 kg/m²		Prolonged sitting		FMD - semi-recumbent popliteal artery, base diameter, blood flow, SRAUC, and MAP
Vranish et al. 2017 [36]*	12 W; age: 20 ± 0 yr	Quasi-experimental	Duration: 180 min	0 and 180	
	BMI: 24.0 ± 0.8 kg/m²*		Meal: none		
	healthy, recreationally active				
	18 M; age, 24.2 ± 4.4 yr		Prolonged sitting		FMD - supine popliteal artery, base diameter, and blood flow
Vranish et al. 2018 [26]	BMI: 23.6 ± 3.4 kg/m²	Quasi-experimental	Duration: 60 min	0, 30, and 60	
	healthy, inactive		Meal: none		
	8 M 4 W; age, 26.1 ± 1.1 yr		Prolonged lying down		FMD - supine popliteal artery, base diameter, blood flow, and SRAUC
Walsh et al. 2017 [47]	BMI: 24.6 ± 0.4 kg/m²	Unilateral model	Duration: 180 min	0 and 180	
	healthy, recreationally active		Meal: none		

Data presented as mean ± SD unless stated otherwise: * data presented as mean ± SE. Meal: carbohydrate/fat/protein. Abbreviations: BMI, body mass index; FMD, flow mediated dilation; MAP, mean arterial pressure; MetS, metabolic syndrome; RCT, randomized crossover trial; SB, sedentary behaviour; SRAUC, shear rate area under the curve; T2D, type 2 diabetes.

Table 2. Pooled acute effects of prolonged sitting on primary and secondary lower-limb vascular outcomes and leave-one-out sensitivity analysis.

	Main findings					**Leave-one-out sensitivity analysis**	
	number of	number of				Most benefit	Least benefit
Outcome	studies	participants	Pooled effect (95% CI)	P value	I^2, p value	Pooled effect (95% CI)	Pooled effect (95% CI)
FMD	18	258					
30 min	3	48	-0.22 (-0.66 to 0.22)	0.34	0%, p=0.34	-0.33 (-0.89 to 0.23)[a]	-0.19 (-0.75 to 0.38)[b]
60 min	5	81	-0.43 (-1.16 to 0.31)	0.25	74.5%, p<0.01	-0.70 (-1.33 to -0.07)[b]	-0.44 (-1.38 to 0.58)[a]
120 min	4	55	-0.85 (-1.32 to -0.38)	<0.01	9.9%, p=0.36	-1.09 (-1.65 to -0.52)[c]	-0.92 (-1.58 to -0.26)[a]
180 min	11	159	-1.18 (-1.69 to -0.66)	<0.01	67.8%, p<0.01	-1.26 (-1.89 to -0.63)[a]	-0.93 (-1.32 to -0.55)[d]
+240 min	8	118	-0.27 (-0.78 to 0.25)	0.31	66.90%, p=0.01	-0.40 (-0.92 to -0.12)[e]	-0.13 (-0.62 to 0.0.37)[c]
Blood flow	13	195					
30 min	2	37	-0.49 (-1.01 to 2.0)	0.49	89.3%, p=0.01	NA	NA
60 min	4	64	-0.10 (-0.94 to 0.74)	0.82	82.2%, p=0.01	-0.12 (-1.36 to 1.10)[f]	0.24 (-0.36 to 0.84)[g]
120 min	2	30	-0.36 (-2.41 to 1.70)	0.73	91.4%, p<0.01	NA	NA
180 min	8	115	-1.00 (-1.61 to -0.39)	<0.01	76.0%, p<0.01	-1.00 (-1.70 to -0.31)[h]	-0.76 (-1.24 to –0.28)[d]
+240 min	6	96	-0.05 (-0.92 to 0.83)	0.92	87.6%, p<0.01	-0.31 (-1.17 to 0.0.55)[k]	0.25 (-0.53 to 1.03)[i]
SRAUC	16	223					
30 min	2	30	-0.52 (-0.87 to -0.16)	0.01	0%, p=0.45	NA	NA

60 min	3	47	-0.57 (-1.37 to 0.24)	0.17	86.7%, p=0.01	-0.79 (-2.15 to 0.57)[i]	-0.19 (-0.48 to 0.11)[b]
120 min	3	43	-0.68 (-1.77 to 0.41)	0.14	90.4%, p<0.01	-0.90 (-2.79 to 0.99)[c]	-0.19 (-0.57 to 0.18)[b]
180 min	11	159	-0.77 (-1.01 to -0.54)	<0.01	45.3%, p<0.01	-0.83 (-1.06 to -0.60)[i]	-0.78 (-1.04 to -0.53)[j]
+240 min	7	100	-0.24 (-0.43 to -0.05)	0.01	0%, p=0.28	-0.29 (-0.52 to -0.09)[i]	-0.18 (-0.38 to 0.01)[k]
MAP	10	136					
30 min	5	66	0.13 (-0.11 to 0.37)	0.30	0%, p=0.88	0.18 (-0.08 to 0.44)[l]	0.09 (-0.17 to 0.36)[m]
60 min	5	65	0.12 (-0.12 to 0.36)	0.29	0%, p=0.49	0.19 (-0.07 to 0.46)[n]	0.05 (-0.24 to 0.34)[o]
120 min	6	79	-0.01 (-0.21 to 0.23)	0.90	0%, p=0.50	0.08 (-0.15 to 0.32)[a]	-0.02 (-0.26 to 0.22)[p]
180 min	9	127	0.15 (-0.05 to 0.35)	0.14	21.3%, p=0.26	0.19 (-0.02 to 0.39)[q]	0.08 (-0.11 to 0.27)[d]
+240 min	2	27	-0.05 (-0.42 to 0.33)	0.80	0%, p=0.40	NA	NA

Data are presented as standardised mean change (95% CI). Abbreviations: FMD, flow-mediated dilation; MAP, mean arterial pressure; SRAUC, shear rate area under the curve. [a] omitted Ballard et al. (2017) [38]; [b] omitted Thosar et al. (2014) [25]; [c] omitted Climie et al. (2018) [7]; [d] omitted O'Brien et al. (2019 – women) [45]; [e] omitted Peddie al. (2021) [48]; [f] omitted Vranish et al. (2018) [26]]; [g] omitted Morishima et al. (2016) [9]; [h] omitted Vranish et al. (2017) [36]; [i] omitted Taylor et al. (2020) [55]; [j] omitted Restaino et al. (2016) [10]; [k] omitted Restaino et al. (2015) [23]; [l] omitted Francois et al. (2016 – healthy, active) [42]; [m] omitted Francois et al. (2017 – type 2 diabetes) [42]; [n] omitted Carter et al. (2017) [37]; [o] omitted Credeur et al. (2019) [41]; [p] omitted Francois et al. (2016 – healthy, inactive) [42]; [q] omitted Morishima et al. (2017) [20].