The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-analysis

FRANCES C. TAYLOR[1,2], ANA J. PINTO[3], NIRAV MANIAR[4,5], DAVID W. DUNSTAN[1,2], and DANIEL J. GREEN[6]

[1]Baker Heart and Diabetes Institute, Melbourne, VIC, AUSTRALIA; [2]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, AUSTRALIA; [3]Applied Physiology and Nutrition Research Group, Laboratory of Assessment and Conditioning in Rheumatology, School of Physical Education and Sport, Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, BRAZIL; [4]School of Behavioural and Health Sciences, Australian Catholic University, Melbourne, VIC, AUSTRALIA; [5]Sports Performance, Recovery, Injury and New Technologies (SPRINT) Research Centre, Australian Catholic University, Fitzroy, Victoria, AUSTRALIA; and [6]Department of Exercise and Sport Science, School of Human Sciences, The University of Western Australia, Perth, AUSTRALIA

ABSTRACT

TAYLOR, F. C., A. J. PINTO, N. MANIAR, D. W. DUNSTAN, and D. J. GREEN. The Acute Effects of Prolonged Uninterrupted Sitting on Vascular Function: A Systematic Review and Meta-analysis. *Med. Sci. Sports Exerc.*, Vol. 54, No. 1, pp. 67–76, 2022. **Objective:** This study aimed to determine the dose–response relationship between prolonged sitting and vascular function in healthy individuals and those with metabolic disturbances and to investigate the acute effects, on vascular function, of interventions that target interrupting prolonged sitting. **Design:** This is a systematic review with meta-analysis. **Data Sources:** Ovid Embase, Ovid Medline, PubMed, and CINAHL were searched from inception to 4 December 2020. **Eligibility Criteria:** Randomized crossover trials, quasi-randomized trials, and parallel group trials where vascular function (flow-mediated dilation [FMD]) was assessed before and after an acute period of sedentary behavior was used in this study. **Results:** Prolonged sitting resulted in a significant decrease in the standardized mean change (SMC) for lower-limb FMD at the 120-min (SMC = −0.85, 95% confidence interval [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 standardized mean difference for FMD, relative to prolonged sitting, but it was not statistically significant (0.13, 95% CI = −0.20 to 0.45). **Conclusions:** 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 that prolonged sitting negatively affects 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

Excessive time spent in sedentary behaviors, 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–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 summarized 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 because 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 behavior (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). Although studies indicate interrupting prolonged sitting can episodically improve vascular function (7,17,18), interruption strategies have reported mixed results, likely because of the wide range of methodologies used 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 behavior 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 subgroup 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 with 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 no. CRD42020171394) (27).

Search strategy and study selection. Four electronic databases (Ovid Embase, Ovid Medline, PubMed, and CINAHL) were searched from inception to June 23, 2021 (December 4, 2019; September 23, 2020; and June 23, 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, PA), 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: randomized crossover trials and quasi-experimental.
- Population: adults and older adults (≥18 yr) 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 behavior (1) period >30 min, but <24 h.
- Outcome: FMD was assessed pre- and post-prolonged sedentary behavior and complied with standardized FMD protocol to ensure our protocol was comparable between studies (19). Although 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 affect 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, 60, 120, 180, and ≥240 min of prolonged, uninterrupted sedentary behavior. The following secondary outcomes were assessed before and after at 180 min of prolonged sedentary behavior: 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-intensity 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 behavior (position and duration), FMD assessment (arterial site and 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 normalized to shear rate. If the data were unclear or were not available in the published manuscripts, the corresponding author or the first author was contacted by e-mail 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 standardized mean change (SMC) using pre- and post-prolonged sedentary behavior data, pre-prolonged sedentary behavior 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 model. The first analysis compared the effects of prolonged sedentary behavior (30, 60, 120, 180, 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 min was used. A negative mean change indicated that vascular function was impaired at that time point when compared with baseline (0 min). Subsequently, a subgroup 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. Subgroup 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 behavior, between-group comparisons (prolonged sedentary behavior vs activity interruptions) were calculated as the effect size difference (standardized mean difference) using post-intervention (FMD%), pre-intervention SD, sample size, and pre- to postcorrelation 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 comparisons 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 eight trials were included in the meta-analysis. Significance was set at $P < 0.05$ (two-tailed). Data are presented as SMC or standardized mean difference 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 6203 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 standardized 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 postdeflation 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 (9,10,17,18,20–26,36–49); overweight or obesity, 121 (7,50–52); metabolic syndrome, 5 (53); and T2D, 36 (42,54). 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 [53] to 56 [52]), and most participants were male ($n = 263/395$; 67%). One study did not report the sex of participants [52]. Typically, studies recruited young participants <30 yr (median, 26 yr; range, 20.0 [10] to 61.5 [54]), whereas populations with chronic disease risk factors or clinical conditions were likely to be older (median, 47.9; range, 32.2 yr [50] to 61.5 [54]).

Bouts of prolonged sitting ranged from 0.5 h (44,46) to 8.5 h (54), with the median duration of 3.0 h. The majority of study protocols assessed the effect of prolonged sitting on vascular function; however, three studies utilized prolonged laying down (44,47,50) and two studies used undefined sedentary behavior (40,52). Assessments of FMD were mostly performed in the supine position (9,10,18,20–23,26,37,39,41,42,44,46–51), with only eight studies performing (49) FMD in the seated position (7,17,24,25,38,45,53,54) and one study in a semirecumbent position (36). Vascular function was predominantly assessed in the lower limb ($n = 23$; superficial femoral artery, 8 studies
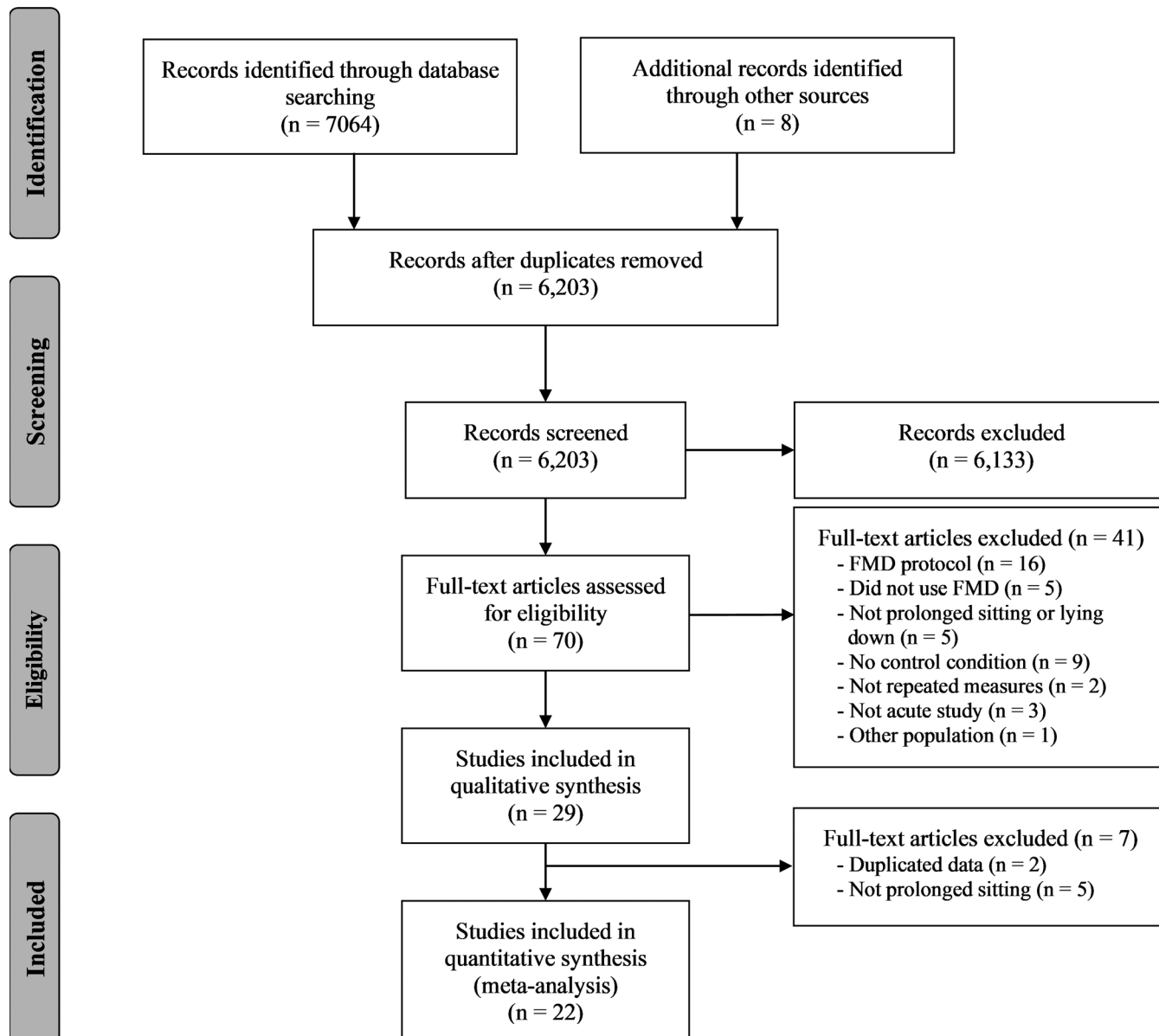
FIGURE 1—Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of the literature search results.

[7,17,18,24,25,38,46,54]; popliteal artery, 14 studies [9,10, 20–23,26,36,43,45,47–49,51]; posterior tibial artery, 1 study [41]), with only 12 trials assessing the brachial artery (7,23,25,37,39,40,42–44,50,52,53). 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 ($n = 2$) (7,54), walking ($n = 3$) (17,18,48), stair sprints (49), and calisthenics ($n = 1$) (37). Experimental protocols lasted between 1.4 h (37) and 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 (54). From the seven 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 ($n = 21$) (54). Sample sizes were <20, except Taylor et al. (2020) (54), and most included participants were male ($n = 73$).

Study quality. The quality score and the 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 randomized, except seven 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 five studies provided details regarding allocation concealment (7,21,40,48,54). Most studies, except two studies (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, 45,48,50,54). Most studies were classified as low risk of bias

for incomplete outcome data. Finally, one study reported not controlling participants leg movement (18), and one 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 (40,44,47,50,52) and 2 reported duplicated data (17,24). Twenty-four 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. Seven 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 Fig. 2; 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; Fig. 2; Supplementary Table 2, http://links.lww.com/MSS/C407) time points. Although no significant differences were observed at the 30-, 60-, or $\geq$240-min time points ($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 to 180 min (see Supplementary Fig. 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- or 120-min time point (see Supplementary Table 2; Supplementary Fig. 3, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Sensitivity analysis at the 240-min time point indicated that the 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 Fig. 2, http://links.lww.com/MSS/C407).

Subgroup analyses for FMD assessment were only performed on lower-limb FMD results at the $\geq$180-min time point. Subgroup analysis indicated that prolonged sitting resulted in a significant decrease in lower-limb FMD in healthy adults (-1.16, 95% CI = -1.75 to -0.58), but not in adults with metabolic disturbances (-0.51, 95% CI = -1.18 to 0.15; see Supplementary Fig. 4, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Prolonged sitting resulted in a significant decrease in lower-limb FMD irrespective of the position in which FMD was assessed (seated position, -1.25; 95% CI = -2.23 to -0.26; and supine position, -0.82, 95% CI = -1.27 to -0.37; see Supplementary Fig. 5, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). Meta-regression indicated a significant positive association between age and SMC for lower-limb FMD ($\beta = 0.04$, 95% CI = 0.01 to 0.07, $P = 0.02$; see Supplementary Fig. 6, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407). However, there was no influence of sex ($\beta = -0.01$, 95% CI = -0.02 to 0.01, $P = 0.39$; see Supplementary Fig. 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;
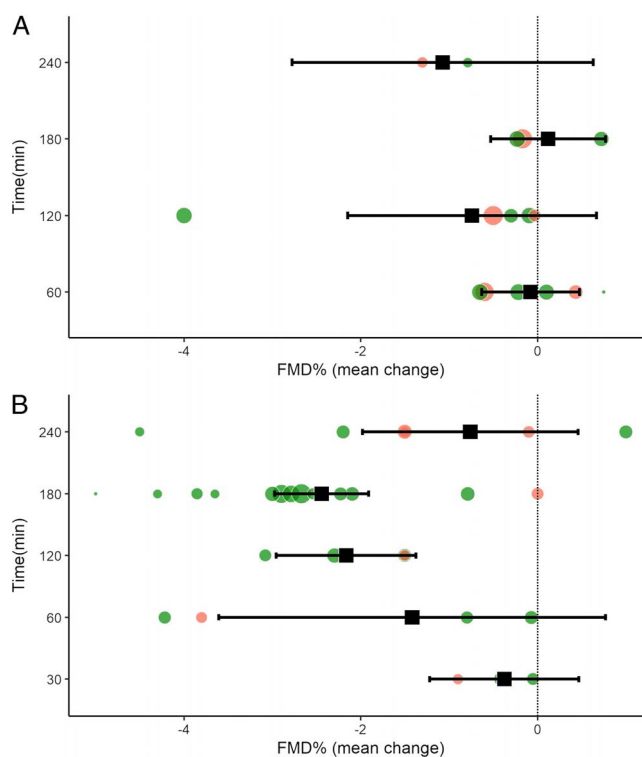
FIGURE 2—Mean time course change for raw FMD% after 30, 60, 120, 180, and $\geq$240 min of prolonged uninterrupted sitting. A, Changes in FMD% in the upper limb. B, Changes in FMD% in the lower limb. *Black square* and *error bars* are presented as mean change (95% CI). *Dots* represent the mean change 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.

Fig. 3), but the result was nonsignificant. Sensitivity analysis indicated that none of the studies were influential to the analysis. A nonsignificant 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 nonsignificant increase for metabolic disturbances (0.29, 95% CI = −0.12 to 0.69). Because of 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 Fig. 9, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C407); however, 180 min revealed some asymmetry (see Supplementary Fig. 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 to 240 min, which may be partially explained by varying methodology (arterial sites measured and posture transitions) and population groups (healthy, metabolic syndrome, and T2D). For lower-limb shear rate, there was considerable heterogeneity at the 60- 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 affect upper-limb function. Although no statistically significant decrease was observed at the 30 and 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 nonsignificant 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 that 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 (55), and it is recognized as the key physiological stimulus in maintaining endothelial health (56). The progressive reduction in lower-limb shear stress from 30 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). Although 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 (55,57,58). Reduced muscle activity and increased pressure in the back of the thighs are also likely contributors (59). Indeed, lower-limb shear rate decreased at all time points relative to presitting 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.
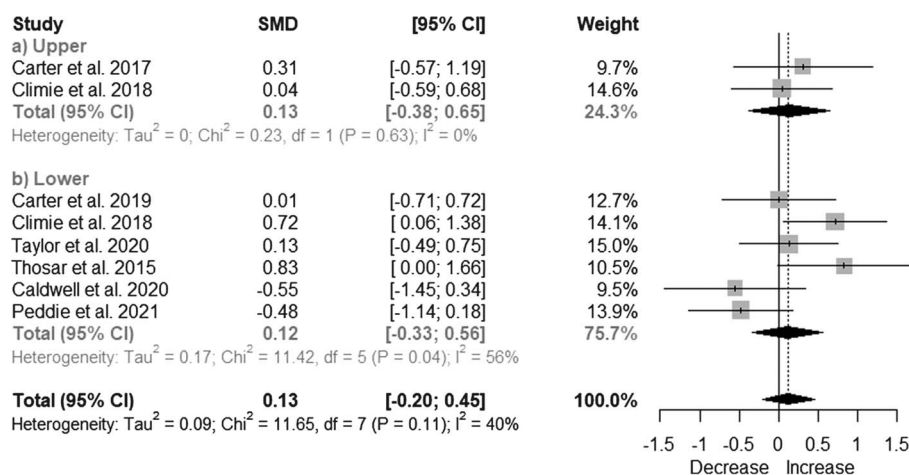

FIGURE 3—Effects of active interruptions on vascular function (FMD) meta-analysis using a random-effects model grouped by upper- and lower-limb assessment. SMD, standardized mean difference.

Meta-analyses have observed similar findings in healthy populations after 180 min of sitting (8). Maintenance of shear stress by continuation of desk-based activities while 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 time points. Although 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 (60,61). The lack of change in upper-limb vascular function after prolonged sitting may reflect the relatively modest effects on shear rate associated with sitting. Reduced lower-limb shear may affect brachial artery vascular function over days, weeks, months, and years. Indeed, bed rest studies assessing vascular function over multiple weeks have demonstrated sedentary behavior to be a strong stimulus for rapid structural remodeling 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 the lower limbs.

Subgroup analyses. For time points 60–180 min (Fig. 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 (12,62). Although the data in this study are reflective of acute changes, repeated exposures to this is additionally concerning for high-risk and clinical populations, where reduced vascular function can further compound preexisting cardiovascular risk factors such as older age, obesity, and T2D (63). Further, older individuals and those with metabolic disturbances typically spend more time in sedentary behavior relative to young and healthy individuals (15,64–67), further contributing to their cardiovascular disease 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 effect of prolonged uninterrupted sitting is imperative to quantify prolonged sitting thresholds and inform public health recommendations.

We observed that prolonged sitting had less apparent effect on vascular function in older adults and those with metabolic disturbances than in young healthy subjects. This may reflect the law of initial values (68). 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 (10,17,21–26,38,49,53), and an additional 13 studies recruited majority male participants (7,9,18,20,37,41, 42,44,46–48,51,54). Given many studies have reported excessive sitting time in female populations from young, healthy females (69) to older females experiencing cardiovascular complications (70), it is necessary to increase female participant recruitment in the area of vascular function and sedentary behavior. It is conceivable that sex differences may be present in the effect of prolonged sitting, given the well-described hormonal effects on endothelial function (71). Continued failure to recruit females, older adults, and individuals with preexisting cardiovascular risk factors not only widens the existing gap in the literature but also 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). Although many studies assumed the supine position during assessment, eight of the studies measured vascular function while sitting. Subanalysis 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. Although 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 utilize 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 (25,54). Future research is needed to assess the validity and reliability of seated FMD measures.

Interrupting sitting with activity. Despite growing evidence indicating that reducing and interrupting prolonged sitting time positively influences glucose metabolism and shear stress profiles across the metabolic risk spectrum (72,73), limited research exists investigating the effects of regularly interrupting sitting on vascular function (8). Our meta-analysis included seven trials and demonstrated a nonsignificant increased FMD response (0.13, 95% CI = −0.20 to 0.45) when sitting was regularly interrupted with activity compared with 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 behavior.

Recent meta-analyses have demonstrated similar improvements in both short- and long-term sedentary behavior interventions (8,74). 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

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disturbances benefit from an increase in FMD after 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- 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 min (7), future research should consider measuring vascular function at earlier time points. Second, 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.

CONCLUSIONS

Prolonged uninterrupted sitting progressively impairs lower-limb vascular function up to 180 min. In healthy populations, vascular function may be more susceptible to the effects 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 are 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 min apart.

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