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REVIEW

Prolonged sitting and peripheral vascular function: potential mechanisms and methodological considerations

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Abstract

Sitting time is associated with increased risks for subclinical atherosclerosis and cardiovascular disease development, and this is thought to be partially due to sitting-induced disturbances in macro- and microvascular function as well as molecular imbalances. Despite surmounting evidence supporting these claims, contributing mechanisms to these phenomena remain largely unknown. In this review, we discuss evidence for potential mechanisms of sitting-induced perturbations in peripheral hemodynamics and vascular function and how these potential mechanisms may be targeted using active and passive muscular contraction methods. Furthermore, we also highlight concerns regarding the experimental environment and population considerations for future studies. Optimizing prolonged sitting investigations may allow us to not only better understand the hypothesized sitting-induced transient proatherogenic environment but to also enhance methods and devise mechanistic targets to salvage sitting-induced attenuations in vascular function, which may ultimately play a role in averting atherosclerosis and cardiovascular disease development.

macrovascular function; microcirculation; peripheral arterial disease; prolonged sitting; shear stress

INTRODUCTION

Sedentary behaviors, particularly time spent in the seated position, have been on the rise in the United States. Adults and children alike currently spend a substantial amount of their daily lives seated, with adults sitting for 6–8 h per day and youths totaling nearly 7 h per day (1–4). With our nation being heavily reliant on technology for work and leisure purposes, it is plausible to expect that time in the seated position may persist or potentially escalate. This is concerning as sitting time has been identified as an independent risk factor for the manifestation of metabolic impairments, cardiovascular diseases, and all-cause mortality (5, 6).

Prolonged sitting, identified as sitting uninterrupted for 1 h or more at a time (7, 8), places unique challenges on the cardiovascular system, with a recent meta-analysis and a review both indicating that substantial vascular impairments may occur in the lower extremities following a prolonged sitting bout (9, 10). It has been suggested that these cardiovascular perturbations may be partially due to "arterial bending" at the hip and knee joints and elevations in hydrostatic pressure that alter local hemodynamics, collectively contributing to sitting-induced disturbances in macro- and microvascular function as well as circulating molecules (7, 8, 10–15). Importantly, impairments in the macro- and microcirculation are classified as contributing factors in the development of lower-extremity atherosclerotic diseases (16–18), and sitting time alone was recently identified to be linked to subclinical atherosclerosis and lower-extremity vascular disease (19, 20). Therefore, preventing sitting-induced disruptions in vascular function may be an area of interest to reduce risks for atherosclerosis.

Prophylactic exercise strategies and breaking-up prolonged sitting with skeletal muscle contractions with activities such as walking (21), fidgeting (22), cycling/pedaling (8, 23), simple resistance exercises (24, 25), and passive leg movements (8) have been shown to have potential protective effects against sitting-induced disturbances in vascular function in healthy and disease populations. These findings are reasonably promising, as averting sitting-induced alterations in vascular function may yield a considerable amount of protection against developing lower-extremity atherosclerosis. However, the mechanism(s) underlying this vascular protection during prolonged sitting is not fully understood. Therefore, this review will focus on *1*) the potential mechanisms of sitting-induced alterations in peripheral hemodynamics including macrovascular function, microvascular function, and circulating molecular imbalances, *2*) potential protective mechanisms by muscular contractions against prolonged sitting-induced disturbances in vascular function and circulating molecules, and *3*) suggestions regarding the experimental environment and study populations for future work.

IMPACTS OF PROLONGED SITTING ON PERIPHERAL HEMODYNAMICS

Hemodynamics refers to the distribution and forces of blood flow throughout the circulatory system. The vascular

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endothelium is continuously subjected to the frictional forces produced by arterial blood flow, otherwise known as shear stress (26, 27). The endothelium reacts to these forces and conveys information via biochemical signals to regulate the phenotypic changes required for maintaining vascular homeostasis (26, 27). These endothelial-mediated responses are contingent on the nature of the shear stress patterns. Unidirectional laminar shear stress stimulates the synthesis of antiatherogenic vasodilatory molecules such as nitric oxide (NO) and prostacyclin (28). On the contrary, regions of disturbed laminar shear stress stimulate production of potent proatherogenic substances and molecules such as endothelin-1 (ET-1) and vascular adhesion molecules (28). Furthermore, low arterial shear stress can upregulate endothelial-derived reactive oxygen species (ROS) and can blunt endogenous antioxidant mechanisms, which may generate a pro-oxidative shift in the redox balance (29, 30). Atherosclerotic plaques have been reported to commonly manifest near arterial bifurcations, and this may be partly due to the bifurcation's structural complexity that fosters an unfavorable local hemodynamic environment that is associated with vasoactive metabolite imbalances, enhanced inflammatory signaling, and greater ROS (29–34). It may be possible that repetitive and/or prolonged exposure to local hemodynamic impairments could contribute to lower-extremity atherosclerotic diseases (10). In particular, the seated position may mimic a similar environment to arterial bifurcations due to the "bent artery" morphology created by 90-degree angles at the hip and knee joints. Walsh et al. (11) revealed that 3 h of leg bending, similar to the angulation of the seated position, produces hemodynamic disturbances in the popliteal artery when compared with a straight limb (Fig. 1A). Therefore, the "bent artery" position associated with the seated posture may be a key contributor to sitting-induced changes in hemodynamics.

Hemodynamic impairments due to prolonged sitting have been well reported. Thosar et al. (21, 35) performed two studies that investigated the impacts of prolonged sitting (3 h) in healthy young males on superficial femoral artery hemodynamics using Doppler ultrasound (Table 1). The authors showed reductions in the superficial femoral artery mean and antegrade shear rates after just 1 h of sitting (21, 35). In line with these investigations, we found reduced common femoral artery mean shear rate and blood flow after prolonged sitting for 2.5 h (8). Furthermore, sitting-induced hemodynamic alterations are consistently present in other downstream arteries, including reduced shear rate and blood flow in the popliteal and posterior tibial arteries (14, 36, 38, 39) (Table 1). Although these findings are largely in agreement, it is important to note that shear rate provides an estimation of shear stress, which is a limitation for Doppler ultrasound. A recent study by Hou et al. (13) used computational modeling techniques to understand how the arterial deformation due to the seated position (i.e., arterial bending) can impact external iliac artery shear stress in vivo (Table 1). After 30 min in a seated position, a greater area of the external iliac artery was exposed to higher oscillatory shear index, low shear stress, and alterations in transverse wall shear stress (indicates the average wall shear stress component over the cardiac cycle that is perpendicular to the mean wall shear stress vector) when compared with a supine position (13). Based on these studies, we can infer that the prolonged leg arterial bending that is associated with the seated position can impair hemodynamics, which may be a factor in producing a potential transient proatherogenic environment (10). Therefore, devising methods that salvage arterial hemodynamics may prevent the formation of this proatherogenic phenotype during prolonged sitting.

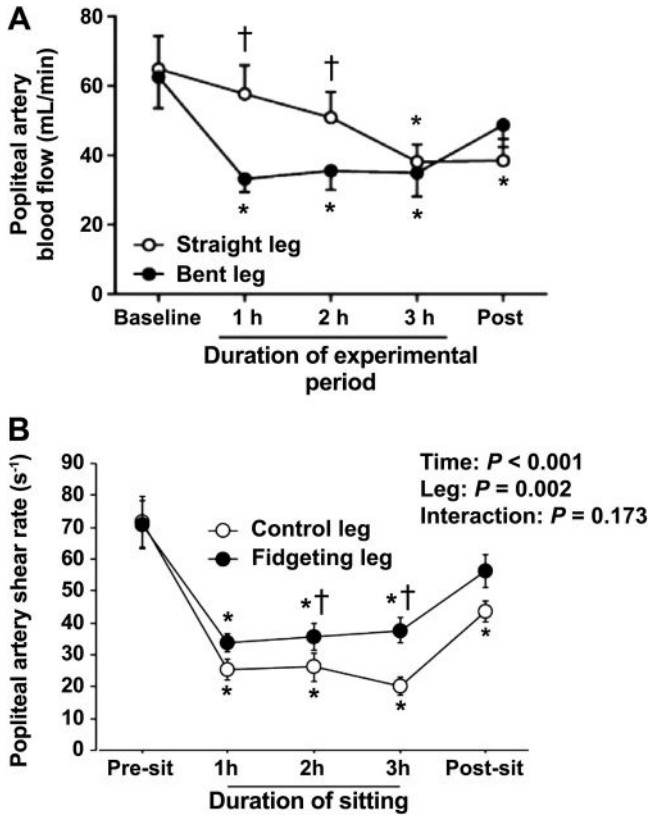

Figure 1. The effects of the bent leg position and intermittent active muscular contractions (fidgeting, 1 min on/4 min off) during prolonged sitting on popliteal artery hemodynamics. *A*: the bent leg position associated with prolonged sitting produces reductions in popliteal artery blood flow when compared with an otherwise straight limb (mL/min), $n = 12$, $^{*}P < 0.05$ vs. Baseline, $^{\dagger}P < 0.05$ vs. straight leg. *B*: mean popliteal artery shear rate (s^{-1}) was greater in the fidgeting leg compared with the nonfidgeting control leg when measured 2 min after the 1-min fidgeting bout. $n = 11$, $^{*}P < 0.05$ vs. baseline $^{\dagger}P < 0.05$ vs. control leg. [Adapted and reused with permission from Walsh et al. (11), under a Creative Commons Attribution 4.0 International License, and from Morishima et al. (22).]

Our group and others suggested that prolonged sitting-mediated increases in venous pooling may be a potential target for preventing these hemodynamic disturbances (7, 8, 37–40), but recent work has revealed that addressing venous pooling alone is not sufficient for hemodynamic preservation (37). Horiuchi and Stoner (37) performed a study that prevented sitting-induced venous pooling with compression stockings, but these stockings were not capable of averting sitting-induced reductions in leg arterial blood flow. This indicates that methods should be directly targeted at improving arterial hemodynamics rather than reducing venous pooling alone. Of note, our group and others have used intermittent active skeletal muscle contractions to prevent sitting-induced alterations in hemodynamics during prolonged sitting (8, 21, 22, 24, 25). Morishima et al. (22) showed that intermittent active skeletal muscle contractions (i.e., "fidgeting" 1 min on/4 min off) could produce higher levels of mean popliteal artery shear rate when compared with the

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Table 1. *Impacts of uninterrupted prolonged sitting on peripheral hemodynamics, macrovascular function, microvascular function, and vasoactive molecules*

References	Sex and Age of Subjects, yr	Sitting Duration, h	Location	Outcomes
		Peripheral hemodynamics		
Credeur et al. (36)	M and F, 26 ± 7 (n = 20)	3	Posterior tibial artery	↓ Mean shear rate ↓ Oscillatory shear index
Horiuchi et al. (37)	M and F, 22 ± 4 (n = 20)	3	Popliteal artery	↓ Blood flow
Hou et al. (13)	M, 23 ± 2 (n = 5)	0.5	External iliac artery	↑ Oscillatory shear index ↓ Retrograde flow ↓ Shear stress
Park et al. (8)	M and F, 24 ± 2 (n = 14)	2.5	Common femoral artery	↓ Mean shear rate ↓ Blood flow
Restaino et al. (38)	M, 27 ± 1 (n = 11)	6	Popliteal artery	↓ Mean blood flow ↓ Mean blood velocity ↓ Mean shear rate
Restaino et al. (39)	M, 26 ± 1 (n = 10)	3	Popliteal artery	↓ Mean shear rate ↓ Blood flow
Shvartz et al. (40)	M, 25 (n = 8)	5	Thigh and calf	↔ Thigh blood flow ↓ Calf blood flow
Thosar et al. (35)	M, 24 ± 4 (n = 12)	3	Superficial femoral artery	↓ Antegrade shear rate ↓ Mean shear rate ↔ Retrograde shear rate
Thosar et al. (21)	M, 24 ± 4 (n = 12)	3	Superficial femoral artery	↓ Antegrade shear rate ↓ Mean shear rate ↔ Retrograde shear rate
Walsh et al. (11)	M and F, 26 ± 1 (n = 12)	3	Popliteal artery	↓ Mean shear rate ↓ Antegrade shear rate ↑ Retrograde shear rate
		Macrovascular function		
Barone Gibbs et al. (41)	M and F, 42 ± 12 (n = 25)	10	Peripheral and central PWV	↔ Carotid-femoral PWV ↔ Carotid-radial PWV ↑ Carotid-ankle PWV
Climie et al. (25)	M and F, 57 ± 12 (n = 19)	5	Superficial femoral artery FMD	↓ Superficial femoral artery FMD
Credeur et al. (36)	M and F, 26 ± 7 (n = 20)	3	Posterior tibial artery FMD and central PWV	↑ Carotid-femoral PWV ↓ Posterior tibial FMD
Evans et al. (42)	M and F, 22 ± 3 (n = 20)	3	Central PWV	↔ Carotid-femoral PWV
Headid et al. (7)	M and F, 22 ± 2 (n = 12)	2.5	Popliteal artery FMD, peripheral and central PWV	↓ Popliteal artery FMD ↔ Carotid-femoral PWV ↔ Carotid-radial PWV ↔ Carotid-distal PWV
Morishima et al. (22)	M and F, 26 ± 1 (n = 11)	3	Popliteal artery FMD	↓ Popliteal artery FMD
Morishima et al. (15)	M and F, 27 ± 1 (n = 15)	3	Popliteal artery FMD	↓ Popliteal artery FMD
O'Brien et al. (43)	M and F, 24 ± 3 (n = 20)	3	Popliteal artery FMD	↓ Popliteal artery FMD
Park et al. (8)	M and F, 24 ± 2 (n = 14)	2.5	Popliteal artery FMD, peripheral and central PWV	↓ Popliteal artery FMD ↔ Carotid-femoral PWV ↔ Carotid-radial PWV ↔ Carotid-ankle PWV ↔ Femoral-ankle PWV
Restaino et al. (38)	M, 27 ± 1 (n = 11)	6	Popliteal artery FMD	↓ Popliteal artery FMD
Restaino et al. (39)	M, 26 ± 1 (n = 10)	3	Popliteal artery FMD	↓ Popliteal artery FMD
Taylor et al. (24)	M and F, 62 ± 8 (n = 24)	7	Femoral artery FMD	↔ Femoral artery FMD
Thosar et al. (35)	M, 24 ± 4 (n = 12)	3	Superficial femoral artery FMD	↓ Superficial femoral artery FMD
Thosar et al. (21)	M, 24 ± 4 (n = 12)	3	Superficial femoral artery FMD	↓ Superficial femoral artery FMD
		Microvascular function		
Clime et al. (25)	M and F, 57 ± 12 (n = 19)	5	Femoral artery reactive hyperemia	↔ Shear rate AUC
Credeur et al. (36)	M and F, 26 ± 7 (n = 20)	3	Posterior tibial artery reactive hyperemia	↓ Posterior tibial artery AUC
Decker et al. (44)	M and F, 24 ± 4 (n = 26)	1.5	Passive leg movement	↓ Common femoral artery peak blood flow ↔ Common femoral artery blood flow AUC
Garten et al. (23)	M and F, 57 ± 1 (n = 10)	3	Passive leg movement	↓ Common femoral artery blood flow AUC
Headid et al. (7)	M and F, 22 ± 2 (n = 12)	2.5	Medial gastrocnemius reactive hyperemia	↓ Tissue oxygenation index recovery rate
Horiuchi et al. (45)	M and F, 21 ± 2 (n = 20)	3	Medial gastrocnemius reactive hyperemia	↓ Tissue oxygenation index AUC ↓ Tissue oxygenation index recovery rate
Kurosawa et al. (46)	M, 23 ± 1 (n = 9)	8	Lateral gastrocnemius tissue oxygenation	↓ Oxygenated hemoglobin ↓ Total hemoglobin

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Table 1.— *Continued*

References	Sex and Age of Subjects, yr	Sitting Duration, h	Location	Outcomes
O'Brien et al. (43)	M and F, 24 ± 3 (*n* = 20)	3	Popliteal artery reactive hyperemia	↓ Shear rate AUC
Park et al. (8)	M and F, 24 ± 2 (*n* = 14)	2.5	Medial gastrocnemius reactive hyperemia	↓ Tissue oxygenation index recovery rate
Restaino et al. (38)	M, 27 ± 1 (*n* = 11)	6	Popliteal artery reactive hyperemia	↓ Popliteal mean blood flow AUC ↓ Popliteal artery shear AUC
Restaino et al. (39)	M, 26 ± 1 (*n* = 10)	3	Popliteal artery reactive hyperemia	↓ Blood flow AUC ↓ Shear rate AUC
Vranish et al. (47)	M and F, 23 ± 4 (*n* = 18)	3	Popliteal artery reactive hyperemia	↓ Popliteal artery blood velocity AUC
			Vasoactive molecules	
Ballard et al. (48)	M, 21 ± 2 (*n* = 11)	3	Plasma oxidant status and endothelin-1	↔ Malondialdehyde ↔ Endothelin-1
Climie et al. (25)	M and F, 57 ± 12 (*n* = 19)	5	NO bioavailability, endothelin-1, and inflammatory makers	↑ Endothelin-1 ↔ Plasma nitrate/nitrite ↔ Intracellular adhesion molecule-1 ↔ Vascular cell adhesion molecule-1
Decker et al. (44)	M and F, 24 ± 4 (*n* = 26)	1.5	Whole blood superoxide	↔ Superoxide concentration
Evans et al. (49)	M and F, 22 ± 3 (*n* = 20)	3	Endothelial microparticles, circulating angiogenic cells, endothelin-1	↓ Endothelial microparticles ↔ Circulating angiogenic cells ↔ Endothelin-1
Garten et al. (23)	M and F, 57 ± 1 (*n* = 10)	3	Plasma oxidant status and inflammatory markers	↔ Malondialdehyde ↔ Superoxide dismutase ↔ Interleukin-6
Park et al. (8)	M and F, 24 ± 2 (*n* = 14)	2.5	NO bioavailability and endothelin-1	↓ Total plasma nitrate/nitrite ↔ Endothelin-1 ↓ Total plasma nitrate/nitrite to endothelin-1 ratio
Taylor et al. (24)	M and F, 62 ± 8 (*n* = 24)	7	Endothelin-1	↔ Endothelin-1

AUC, area under the curve; F, female; FMD, flow-mediated dilation; M, male; PWV, pulse-wave velocity. ↑, increase; ↓, decrease; ↔, no change.

nonfidgeting control limb during 3 h of prolonged sitting (Fig. 1*B*). We hypothesize that a key contributing mechanism underlying these protective effects is muscle afferent activation. Group III (mechanosensitive) and group IV (metabosensitive) afferents are the major mechanisms that stimulate central and peripheral cardiovascular responses during skeletal muscle contraction (50, 51). Previous human studies have used passive leg movements to activate group III afferents and active leg movements to activate both group III and IV afferents (52, 53). We recently applied both passive and active leg movement stimuli to break up prolonged sitting in healthy young adults, and we found that active leg movements could preserve arterial hemodynamics while passive leg movements only could induce modest protective effects (8). We concluded that group IV afferent activation, prompted by the metabolites produced during active muscular contractions, is required to preserve arterial hemodynamics during prolonged sitting. However, investigations regarding the contribution of local skeletal muscle metabolites (e.g., potassium, adenosine, hydrogen ions, etc.), by methods such as intramuscular metabolite injection with postexercise circulatory occlusion (PECO), are warranted to fully confirm group IV afferent activation.

Although most of the sitting literature primarily attributes hemodynamic alterations to the hypothesized "bent artery" morphology, we and others have also proposed that activation of muscle sympathetic nerve activity (MSNA) may be an interesting contributing mechanism (7, 8, 10, 21). Postural changes from the supine to the seated position have been reported to increase MSNA in adults (54), and Padilla et al. (55) showed that increases in leg MSNA are associated with perturbations in shear rate patterns, such as greater retrograde shear rate and oscillatory shear index. Considering the relationship between MSNA with the seated posture and hemodynamic alterations, we and others have speculated that increased MSNA may play a role in hemodynamic disturbances, reduced leg blood flow, and increased peripheral vascular resistance during prolonged sitting (7, 8, 10, 38). Interestingly, low-intensity skeletal muscle contractions by cycling have been shown to reduce MSNA (56). This may imply that utilizing intermittent low-intensity skeletal muscle contractions during prolonged sitting may increase laminar shear stress and/or reduce turbulent flow, retrograde flow, and the contribution of MSNA during sitting, which may concomitantly protect against sitting-induced hemodynamic disturbances (56). Future investigations should incorporate use of MSNA and lower extremity hemodynamics to understand the complex interplay of these factors during prolonged sitting both with and without intermittent skeletal muscle contractions, and the intensity of the intermittent skeletal muscle contractions, similar to the fidgeting, calf raises, and cycling from previous work, should be considered (8, 22, 42). In addition to MSNA, future studies should consider investigating cardiovagal baroreflex sensitivity, which may give additional insight into autonomic nervous system function. O'Brien et al. (57) demonstrated that breaking up sedentary time is a stronger predictor of cardiovagal baroreflex sensitivity than total sedentary time alone, which may make cardiovagal baroreflex sensitivity an important addition to understanding prolonged sitting with and without activity breaks on autonomic regulation.

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IMPACTS OF PROLONGED SITTING ON MACROVASCULAR FUNCTION

The macrocirculation includes large arteries and veins, which transport and distribute blood throughout the body. The major functional components of these vessels include the endothelium and the smooth muscle. Under normal physiological conditions, the endothelium responds to hemodynamic alterations and produces vasoactive signaling molecules, such as NO and ET-1 among others, which act on both the endothelium and the smooth muscle to regulate vascular tone (i.e., vasodilation and vasoconstriction) (26, 27, 58). Endothelial and smooth muscle structure and function are known to be altered in disease conditions, such as atherosclerosis, which can severely compromise macrovascular homeostasis.

Although the mechanisms underlying macrovascular dysfunction are not well established, endothelial dysfunction is often characterized by vasoactive molecule dysregulation, excessive ROS, and upregulated inflammatory signaling cascades (59). Furthermore, these factors may also induce structural and functional changes at the level of the vascular smooth muscle such as calcification, changes in extracellular matrix proteins (elastin and collagen), and reduced arterial compliance (60). Even though these changes in the smooth muscle are oftentimes chronic and manifest over several years, alterations in endothelial function can be detected before evidence of atherosclerotic lesions, which suggests that endothelial dysfunction may be a key contributor to the early development of atherosclerotic diseases (61–63). Interestingly, acute bouts of prolonged sitting just a few hours in duration have been found to impair macrovascular endothelial function, most particularly in the lower extremities (7, 8, 21, 22, 35, 36, 38, 39, 43) (Table 1). Considering that endothelial dysfunction has been classically referred to as a "hallmark" of cardiovascular diseases and atherosclerosis, we hypothesize that persistent exposure to sitting-induced attenuations in macrovascular endothelial function may be a factor driving the relationship between sitting time and cardiovascular disease development (5, 6).

Flow-mediated dilation (FMD) is considered the "gold standard" noninvasive assessment for endothelial function, which has been the primary assessment used by the prolonged sitting literature to evaluate macrovascular endothelial function in the upper and lower extremities. Briefly, FMD is performed using a Doppler ultrasound system to assess the change in arterial diameter (% change from initial baseline diameter) in response to an increase in arterial shear stress following a short period of arterial occlusion (i.e., reactive hyperemia) (64, 65). We and others have consistently shown that an acute bout of prolonged sitting attenuates FMD in the superficial femoral artery, popliteal artery, and posterior tibial artery (7, 8, 15, 22, 36, 38, 39, 43). It is hypothesized that these sitting-induced impairments in endothelial function may render the lower extremities more susceptible to atherosclerosis, especially when considering that individuals spend a substantial amount of their waking hours in the seated position (1–4, 10).

Impaired local hemodynamic-induced changes in circulating molecules and cellular signaling factors have been commonly proposed as key contributors to sitting-induced attenuations in lower extremity endothelial function (7, 8, 22, 35, 38, 39). As discussed, the seated posture is associated with greater amounts of low and oscillatory shear rates in the lower extremity conduit arteries (14, 21, 35, 36, 38, 39, 66). Previous work has shown that oscillatory and low shear stress can increase endothelial cell-derived ROS and downregulate endothelial nitric oxide synthase (eNOS) expression and NO production (29, 30, 67). On the contrary, laminar shear stress can theoretically oppose these effects by stimulating upregulation of endothelial antioxidant mechanisms, eNOS-derived NO production, and reductions in vasoconstrictor and pro-inflammatory molecules (67–71). Human in vivo work has been in support of these factors, as experimentally induced increases in brachial artery oscillatory and retrograde shear rates (by forearm cuff compression of 25–75 mmHg) have been shown to attenuate conduit artery endothelial function, likely due to imbalances in endothelial-derived vasoactive substances and redox-related mechanisms (72, 73). Therefore, determining methods that produce favorable flow and shear stress profiles during prolonged sitting may serve to protect against sitting-induced decrements in macrovascular endothelial function, which may provide additional insight into shear-mediated mechanisms, such as changes in molecular and signaling factors, underlying endothelial dysfunction.

Investigations have primarily used local heating and sitting interruptions with intermittent active muscular contractions to increase local muscular metabolism and upregulate favorable shear stress profiles that ultimately preserve macrovascular endothelial function during prolonged sitting (8, 21, 22, 24, 25, 39). For instance, we revealed that intermittent active muscular contraction is required to protect macrovascular endothelial function during prolonged sitting, which may be partially due to upregulated local muscular metabolism and arterial shear stress (Fig. 2*A*), with reduced MSNA or improvements in cardiovagal baroreflex sensitivity also being potential contributing factors (8). However, the mechanisms underlying the protective effects of increased mean and antegrade shear rates on endothelial function have remained poorly investigated. Padilla and Fadel (10) suggested that increases in ET-1 concentrations may be a key contributor to sitting-induced reductions in endothelial function. They hypothesized that increased ET-1 concentrations may upregulate ROS and reduce total plasma nitrate and nitrite, markers of NO bioavailability, and they proposed that these ET-1 concentrations can be reduced when the endothelium is exposed to laminar shear stress (10, 74). In support of this notion, Climie et al. (25) found that resistance exercises performed for 3 min every 30 min during prolonged sitting can attenuate sitting-induced increases in ET-1 concentrations, but no changes in plasma NO bioavailability were noted. In agreement with their findings, we found that active pedaling on an under-desk foot elliptical for 2 min every 30 min during prolonged sitting can reduce ET-1 concentrations but does not change NO bioavailability (8). Furthermore, we reported that these reductions in ET-1 produced a favorable shift in the vasodilator-to-vasoconstrictor balance when compared with control prolonged sitting (no muscular contractions) (8). We hypothesized that intermittent increases in mean arterial shear rate allowed for the preservation of an antiatherogenic phenotype (i.e., preserved vasodilator-to-vasoconstrictor balance). Collectively,

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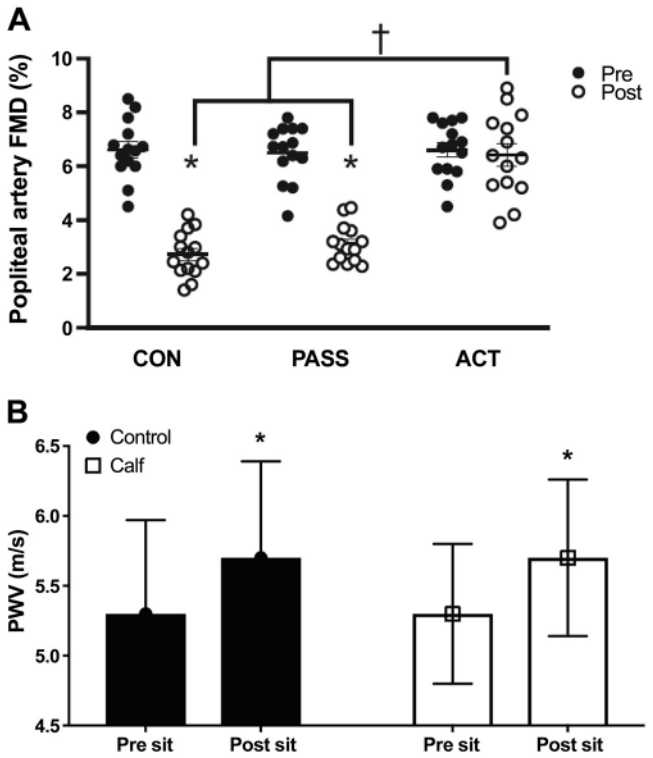

Figure 2. Measurements of popliteal artery flow-mediated dilation (FMD) pre- and postprolonged sitting in a control group (CON, no movement), passive leg movement group (PASS, passive leg movements every 30 min for 2 min at 1 Hz), and active leg movement group (ACT, active leg movements every 30 min for 2 min at 1 Hz at a 13-W workload) and central arterial stiffness [assessed by carotid-to-femoral pulse-wave velocity (PWV)] in response to 3 h of prolonged sitting both with and without intermittent active skeletal muscle contractions (i.e., 10 calf raises at 0.33 Hz every 10 min). *A*: FMD decreased after sitting in CON and PASS but was preserved in ACT. $n = 14$, $^*P < 0.05$ vs. Pre, $^\dagger P < 0.05$ vs. ACT. *B*: carotid-to-femoral PWV (m/s) increased after sitting in both conditions. $n = 20$, $^*P < 0.05$ vs. Pre sit. [Adapted and reused with permission from Park et al. (8) and Evans et al. (42).]

and in support of the hypothesis by Padilla and Fadel (10), these findings suggest that increased ET-1 concentrations and vasoactive substance imbalances may be key mechanisms underlying sitting-induced attenuations in macrovascular endothelial function during prolonged sitting (8, 10, 25).

In addition to sitting-induced changes in macrovascular endothelial function, it is important to consider acute changes in arterial compliance. Arterial stiffness, although a chronic manifestation that is attributed to a large array of pathological sequela (60), has been assessed by pulse-wave velocity (PWV) in response to acute prolonged sitting. Carotid-to-femoral PWV is considered the noninvasive "gold standard" assessment of central arterial stiffness in the field of cardiovascular physiology (75). Carotid-to-femoral PWV has been reported to acutely increase ($\sim \Delta$ 0.3–0.4 m/s) after 3 h of prolonged sitting in young adults (Fig. 2*B*) (36, 42), and carotid-to-ankle PWV, an indicator of peripheral arterial stiffness, has been shown to increase during prolonged sitting in overweight/obese adults with elevated blood pressure (41) (Table 1). These studies suggested that these increases in PWV may be rather trivial (36, 41, 42), as changes in PWV $\geq$ 1.0 m/s may be required to be considered a clinically impactful change (76). However, it has been hypothesized that repetitive exposure to these modest increases in PWV may pose potential cardiovascular consequences (36). Even though we tend to agree with this hypothesis, it is unlikely that structural changes occur due to an acute bout of prolonged sitting, which makes investigation of moment-to-moment changes in potential mechanisms that may have an immediate impact on PWV necessary to understand how prolonged sitting may acutely affect PWV. Future investigations assessing arterial compliance should consider that central and peripheral PWV assessment may be sensitive to these potential changes in MSNA, hemodynamics, blood pressure, and peripheral vascular resistance, which may be of interest to include in forthcoming work.

IMPACTS OF PROLONGED SITTING ON MICROVASCULAR FUNCTION

The primary role of the skeletal muscle microcirculation is to regulate blood flow and oxygen delivery to meet the local metabolic demand (77–80). These processes rely on the complex interplay of the skeletal muscle resistance vessels (i.e., feed arteries and arterioles) and capillary networks. The resistance vessel endothelium responds to biochemical signals to regulate vascular tone and resultant blood flow to the tissue, and capillary networks make appropriate adjustments via recruitment and distension according to local metabolism. Aging and disease negatively impact these regulatory functions of the skeletal muscle microcirculation, and the potential mechanisms by which this dysregulation occurs are multifaceted and poorly understood, with some proposed factors including low-grade inflammation upregulated pro-oxidative signaling cascades (16, 81, 82). We and others hypothesize that repetitive exposure to transient proatherogenic environments, such as prolonged sitting, may foster these unfavorable environments and play a role in impairments in microvascular function and the pathobiology of lower-extremity atherosclerosis (7, 8, 83).

The majority of the prolonged sitting literature has focused on alterations in macrovascular endothelial function, but investigations have shown recent interest in the microcirculation. The microcirculatory response to prolonged sitting has been assessed both indirectly and directly with Doppler ultrasound and near-infrared spectroscopy (NIRS), respectively. Doppler ultrasound has been used to quantify the area under the curve (AUC) for mean blood flow and blood velocity in response to reactive hyperemia in conduit arteries, and this work has demonstrated that microvascular reactivity is blunted after 3–6 h of prolonged sitting in the upper and lower extremities (36, 38, 39, 43, 47, 83) (Table 1). Consistent with these findings, investigations assessing microvascular reactivity with Doppler ultrasound during passive leg movement have revealed that microvascular impairments are present after 1.5–3 h of prolonged sitting (23, 44) (Table 1). Continuous-wave NIRS, which uses near-infrared light to quantify tissue oxygenation status, has been used by our group and others to directly assess the leg microcirculation in response to prolonged sitting (7, 8, 45, 46) (Table 1). These studies revealed impairments in tissue oxygenation index (TOI) recovery rate and TOI AUC during

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reactive hyperemia after 2.5–3 h of sitting (7, 8, 45) and reductions in oxygen delivery to the periphery throughout 8 h of sitting (46).

Our group and others have used active and passive skeletal muscle contractions before, during, and after prolonged sitting in the attempt to prevent or reverse sitting-induced microvascular impairments. Methods such as using a high-intensity exercise session before prolonged sitting (3 h) (23) and a 10-min walk (~1,000 steps) after prolonged sitting (6 h) (38) have been shown to prevent and reverse sitting-induced reductions in microvascular function, respectively. In addition, interrupting prolonged sitting (3 h) with modified squats can protect microvascular function as assessed by the TOI recovery slope and AUC (45). Similarly, we found that interrupting prolonged sitting (2.5 h) with active pedaling on an under-desk foot elliptical can protect the TOI recovery slope (Fig. 3) (8). Furthermore, and unique to our work, we found that intermittent passive leg movements using the same under-desk foot elliptical during prolonged sitting (2.5 h) can also salvage the TOI recovery slope (Fig. 3) (8).

We and others have hypothesized that the protective effects of passive and active muscular contractions on the microcirculation during prolonged sitting are likely due to the complex interplay of several mechanisms, such as favorable shifts in hemodynamic profiles that preserve circulating vasoactive metabolite and redox homeostasis (8, 23, 38, 45). Interestingly, the roles of hemodynamic profiles and vasoactive metabolites show some differences between passive and active muscular contractions. In our previous work, there was a detectable increase in mean arterial shear rate during passive movement bouts, which likely played a role in protecting microvascular function (8). Despite the level of microvascular protection supplied by passive leg movements, active leg movements provided a greater amount (8). This is speculated to be due to the contribution of skeletal muscle metabolism and central hemodynamics (increased blood pressure, heart rate, contractility, etc.) that provoked a more robust increase in mean arterial shear rate compared with passive leg movement, and this greater increase in shear rate is the likely mechanism that preserved circulating NO bioavailability and the vasodilator-to-vasoconstrictor substance balance (8). In line with these findings, Climie et al. (25) found that breaking up prolonged sitting with active muscular contraction can counteract sitting-induced increases in ET-1 concentration, which is a potent endothelial cell-produced vasoconstrictor, and Garten et al. (23) showed that a prophylactic aerobic exercise session preserved passive leg movement-induced hyperemia, which is indicative of maintained NO bioavailability. Interestingly, Garten et al. also noted that both conditions in their study (control and prior exercise bout) were not accompanied by changes in plasma oxidant status, suggesting that oxidative stress may not play an immediate role in sitting-induced attenuations in microvascular function (23). Collectively, these studies suggest that increasing local skeletal muscle metabolism that prompts the release of vasodilators plays an important role in supporting microvascular function and promoting an otherwise antiatherogenic environment (8, 84, 85).

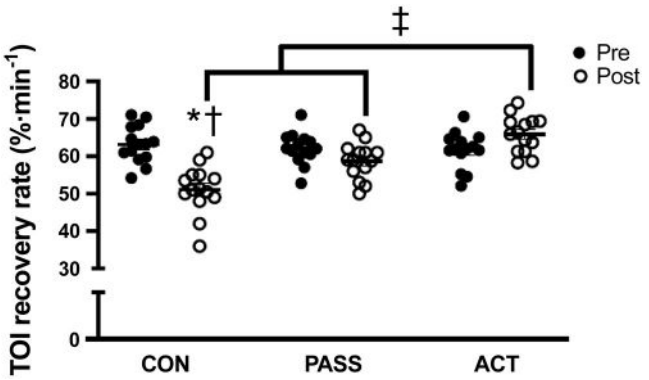

Figure 3. Measurements of microvascular function [tissue oxygenation index (TOI) recovery rate, % min^{-1}] pre- and postprolonged sitting in a control group (CON, no movement), passive leg movement group (PASS, passive leg movements every 30 min for 2 min at 1 Hz), and active leg movement group (ACT, active leg movements every 30 min for 2 min at 1 Hz at a 13 W workload). TOI recovery rate was lower after sitting in CON, and post-CON was less than post-PASS and post-ACT. Post-ACT was greater than post-CON and post-PASS. n = 14, *P < 0.05 vs. Pre, †P < 0.05 vs. PASS, ‡P < 0.05 vs. ACT. [Adapted and reused with permission from Park et al. (8).]

Unfortunately, noninvasive assessments of the microcirculation pose several experimental considerations that must be acknowledged. The conduit artery AUC response (blood velocity and/or flow) to postocclusive reactive hyperemia has been commonly used as a surrogate marker of microvascular function (36, 38, 39, 43, 47, 83), but the analysis process can be extensive with a large margin for error depending on access to edge-detection software and sonography experience (86). Furthermore, the lack of consensus for the most reliable methodological approach for conduit artery AUC (i.e., peak or mean values, normalized values, etc.) poses additional experimental considerations (86). The passive leg movement technique has been used frequently to quantify microvascular function in both healthy and clinical populations (23, 44, 87). The assessment is also considered less technically challenging, thus making it a more attractive method for investigators with less sonography experience and limited software access (87). However, it is important to note that assessments of conduit artery AUC and passive leg movement-induced hyperemia indirectly quantify microvascular reactivity. As such, direct assessments of the microcirculation should be considered for future work. We and others have used NIRS to assess the microvascular response to postocclusive reactive hyperemia and have reported the reperfusion slope (i.e., TOI recovery slope) and TOI AUC (7, 8, 45). Although these have been well-accepted metrics for NIRS, the signals can be altered by adipose tissue thickness, which should be used as a covariate when performing data analyses in future investigations (86). Furthermore, other techniques such as microdialysis, laser Doppler flowmetry, and/or optical coherence tomography should be used in forthcoming studies to provide a more comprehensive and direct assessment of the microcirculation.

IMPACT OF PROLONGED SITTING ON VASOACTIVE MOLECULES

To date, the sitting literature has primarily focused on functional assessments of systemic vascular function. Despite the

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insight provided by these studies, the contribution of molecular mechanisms to these functional deficits remains relatively underexplored. These aspects may be important to consider, as atherosclerosis is associated with alterations in vasoactive metabolites as well as upregulation of proinflammatory and pro-oxidative signaling cascades. Information regarding how prolonged sitting may impact these molecular factors may provide a better understanding of how the hypothesized transient proatherogenic environment is generated by prolonged sitting (7, 8, 83).

We and others have investigated circulating biomarkers from whole blood and plasma samples in response to prolonged sitting, which have included assessments of oxidant status, inflammatory molecules, vasoactive metabolites, endothelial microparticles, and adhesion molecules (Table 1). Thosar et al. (88) and Decker et al. (44) used antioxidant intake (vitamin C, 1,000–1,500 mg) to investigate the potential role(s) of acute oxidative stress during prolonged sitting for 1.5–3 h. They noted that vitamin C intake can ablate sitting-induced alterations in lower extremity microvascular function in males but not in females (44, 88). However, no changes in whole blood superoxide concentrations were noted in response to sitting with or without vitamin C intake (44). Furthermore, studies investigating prolonged sitting (3 h) with and without prior exercise bouts have shown no alterations in markers of plasma redox status (malondialdehyde and superoxide dismutase) or inflammation (interleukin-6) in healthy young males (23, 48). On the contrary, studies investigating circulating vasoactive metabolites during prolonged sitting have shown some inconsistent results in healthy and clinical populations. We and Climie et al. (25) reported that ET-1 concentrations rise during prolonged sitting (5 h) and total plasma nitrate/nitrite levels are lower after prolonged sitting (2.5 h) (8, 25), which resulted in an unfavorable shift in vasoactive metabolite balance (Fig. 4) (8), but Evans et al. (49) and Taylor et al. (24) showed no changes in ET-1 concentrations during prolonged sitting (3–7 h) regardless of intermittent skeletal muscle contractions. In addition, other work has revealed that prolonged sitting (3 h) reduces levels of circulating endothelial microparticles independent of interruptions with active skeletal muscle contractions (49), whereas vascular and cellular adhesion molecules remain unaltered during prolonged sitting (5 h) both with and without sitting interruptions (25).

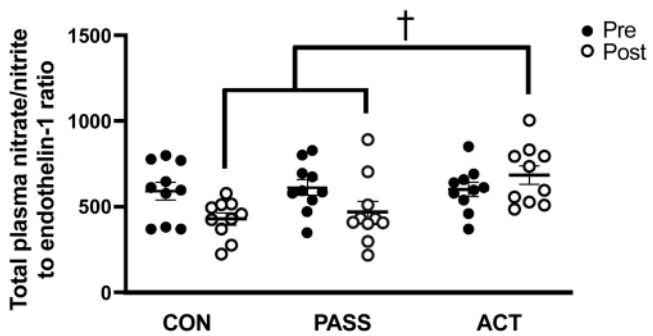

Figure 4. Measurements of total plasma nitrate/nitrite to endothelin-1 ratio pre- and postprolonged sitting in a control group (CON, no movement), passive leg movement group (PASS, passive leg movements every 30 min for 2 min at 1 Hz), and active leg movement group (ACT, active leg movements every 30 min for 2 min at 1 Hz at a 13-W workload). Total plasma nitrate/nitrite to endothelin-1 ratio was lower in post-CON and post-PASS when compared with post-ACT. n = 14, †$P < 0.05$ vs. PASS. [Adapted and reused with permission from Park et al. (8).]

With the limited amount of literature on circulating biomarkers in response to prolonged sitting, there remains a substantial amount of uncertainty regarding their complex interplay. For instance, investigations have focused on markers of inflammation, plasma oxidant status, and vasoactive metabolites, but little work has been performed regarding upstream factors to these molecules. Future work should incorporate greater investigation of endothelial microparticles and vascular adhesion molecules (25, 89), which are known to be associated with elevated cardiovascular disease risk (90). In addition, investigation of other molecular factors may be of-interest, such as microRNAs and extracellular vesicles. Investigations of these factors may provide additional insight into alterations in cellular signaling that may ultimately affect downstream factors pertaining to inflammation, oxidative stress, and/or vasoactive metabolites.

Furthermore, production of these circulating factors in response to prolonged sitting may be altered due to several modifiable and nonmodifiable variables such as age, health status, dietary habits, and physical activity level. For example, healthy young populations often have intact homeostatic regulation even when exposed to low levels of acute physiological stress (e.g., prolonged sitting), which may make it difficult to detect any true changes in these circulating biomarkers. Age and health or disease status may inadvertently affect the pro- and antiatherosclerotic circulating factors that are often assessed in response to prolonged sitting. Although these concerns and concepts have been proposed in brief by our group and others (8, 23, 44, 48, 91), proper execution of these considerations in future studies is paramount to understanding sitting-induced changes in circulating biomarkers of inflammation, oxidative stress, and vascular function.

In conjunction with circulating biomarkers, to our knowledge, only one study has investigated the effects of breaking up prolonged sitting on skeletal muscle gene expression (vastus lateralis) in overweight/obese adults (92). They noted that interrupting prolonged sitting (5 h) with light- and moderate-intensity treadmill walking every 30 min supports increased expression of triglyceride and carbohydrate metabolism-related genes as well as genes that modulate anti-inflammatory and antioxidative cascades (92). Further use of skeletal muscle biopsies, such as biopsies downstream from the vastus lateralis, such as the gastrocnemius, should be included to further investigate the impacts of prolonged sitting on the expression of redox- and inflammatory-relevant genes and proteins, mitochondrial respiration and function, and antioxidant enzyme activity to better characterize prolonged sitting-induced molecular changes at the level of the skeletal muscle.

SEX DIFFERENCES AND OTHER CONSIDERATIONS

The role of sex as a biological variable in the prolonged sitting literature has remained a relatively underinvestigated area. Several of the earlier investigations that have provided a large amount of the groundwork in prolonged sitting

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research only included male participants (21, 35, 39, 88). Recent studies have sought to investigate sex differences, but they have shown some mixed results regarding peripheral macro- and microvascular function. After 3 h of prolonged sitting, Vranish et al. (47) reported that males demonstrated attenuated popliteal artery FMD compared with females, but O'Brien et al. (43) reported no differences between sexes. On the contrary, studies focusing on sex differences in the microcirculation have shown more consistencies in findings. Three studies reported that males and females exhibit similar reductions in microvascular function following 1.5–3 h of prolonged sitting (43, 44, 47). The authors of these studies as well as other reviews on prolonged sitting and cardiovasomobility have discussed some of these sex differences in detail pertaining to sex hormones, which may be an area of interest to explore further (10, 44, 47, 93). Regardless, future studies should power adequately to investigate potential sex differences, especially considering the recent urgency and priority placed on sex as a biological variable in cardiovascular research (94). As such, the age of menarche, menstrual cycle phases, hormonal contraceptive use, as well as other factors specific to female physiology need to be appropriately considered in the design of these studies (95).

Other potential confounding factors to consider in future investigations include habitual physical activity and overall physical fitness level. Short-term reductions in daily physical activity, such as reduced average daily step count for 5–7 days, can profoundly impact popliteal artery FMD, circulating endothelial microparticles, and even skeletal muscle citrate synthase activity (96, 97). Participants should maintain their normal physical activity levels throughout study enrollment, including average daily step count and planned exercise activities, with intense exercise avoided during the 24-h window before each experimental visit. Future work should consider reporting average daily step count (from accelerometers or commercially available fitness trackers) and physical fitness levels (such as true or estimated $\dot{V}O_{2max}$) and/or minutes of moderate-to-vigorous physical activity per week as a component of participant characteristics as we and others have done previously (7, 8, 44).

More adequately resembling day-to-day living conditions should be considered in future work. Most protocols have typically been performed following an overnight fast (7, 8, 21, 35, 42) or after a standardized breakfast (24, 25), whereas other studies have focused on the impacts of prolonged sitting in the postprandial state (prolonged sitting after a standardized meal or meal replacement for participants) on vascular function and metabolic hormone regulation (41, 98, 99). Future work should consider including a standardized meal before and/or snacks during sitting protocols to better reflect day-to-day living habits, especially when experiments last several hours.

Another aspect that should be considered to reflect day-to-day living conditions is the conditions of the investigational environment. Prolonged sitting generally occurs during work and leisure activities in spaces such as shared offices, conference rooms, lecture classrooms, and auditoriums. Oftentimes, these spaces are densely populated and not well ventilated, which causes carbon dioxide (CO_2) produced by natural respiration to accumulate (100). In fact, these levels often climb to nearly four to five times that of normal atmospheric concentrations in these spaces (~1,500 ppm CO_2) in as little as 1–2 h, thus classifying them as "mild hypercapnic environments" (7, 8, 100). Acute exposure to mild hypercapnic conditions alone can place a substantial amount of stress on the cardiovascular and autonomic nervous systems, resulting in alterations in ventilation, heart rate, blood pressure, and hemodynamics. Although these cardiovascular and autonomic responses to a mild hypercapnic environment are otherwise "normal" and warranted to maintain homeostasis, they may generate an additional level of physiological stress that could intensify the negative effects of prolonged sitting on vascular function. We recently reported that a mild hypercapnic environment (~1,500 ppm CO_2) can intensify the negative effects of prolonged sitting on the peripheral circulation in healthy young adults, suggesting that individuals sitting in these conditions may endure greater cardiovascular consequences than the sitting literature has reported thus far (7, 8).

It is important to note that our previous prolonged sitting studies in mild hypercapnic environments were performed in a healthy young adult population (age: ~ 20–26 yr) (7, 8). Our participants averaged ~12,000 steps per day and were "recreationally active" (7, 8). This poses issues with the external validity of our findings, as other populations may be more susceptible to prolonged sitting in these environments. For example, according to the US Bureau of Labor Statistics in 2020, occupations in sectors such as office and administrative support, legal firms, and business and financial operations sit for >75% of their workdays and are on average ~42–46 yr old (middle aged). Considering that sitting time has been identified as an independent risk factor for cardiovascular and metabolic diseases (5, 6), it is only logical to shift the investigational focus to populations who are frequently predisposed to prolonged sitting in these mild hypercapnic environments. Finally, future work should dive deeper into potential contributing mechanisms to sitting-induced alterations in vascular function in these populations to develop novel and efficacious strategies for vascular protection, which may reduce the likelihood of vascular dysfunction and the development of cardiovascular diseases in these at-risk populations.

CONCLUSIONS

Sitting time is an often-overlooked independent behavioral risk factor for the development of cardiovascular diseases and all-cause mortality (5, 6), and we hypothesize that the "bent artery" morphology associated with the seated position is a major initiator of sitting-induced changes in vascular function. With adults and children spending nearly 6–8 h per day in the seated position (1–4), it is important that our field focuses on interventions that are effective at targeting these functional changes. Within the past decade, the sitting literature has revealed that using intermittent skeletal muscle contractions can be effective in negating some of these sitting-induced impairments on the vasculature. However, several gaps in the literature persist. Future prolonged sitting research should *1*) investigate other potential contributing mechanisms to sitting-induced vascular dysfunction and the protective mechanisms of muscular contraction (e.g., MSNA, skeletal muscle metabolites and

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activation of muscle afferents, smooth muscle function, skeletal muscle gene/protein expression and mitochondrial function, etc.), *2*) carefully consider the investigational environment, as many prolonged sitting bouts occur in mild hypercapnic environments that further exacerbate prolonged sitting's negative effects (7), and *3*) focus on populations who may be more at risk for regular bouts of prolonged sitting (e.g., office and administrative support workers, legal professionals, sedentary middle-aged and older-aged individuals, etc.). These future investigations will allow for researchers to optimize therapeutic strategies that prevent sitting-induced vascular dysfunction in populations who may be more susceptible to prolonged sitting, which may play a role in averting metabolic and cardiovascular disease development.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.J.P., M.F.A., and S.-Y.P. prepared figures; E.J.P. and S.-Y.P. drafted manuscript; E.J.P., M.F.A., and S.-Y.P. edited and revised manuscript; E.J.P., M.F.A., and S.-Y.P. approved final version of manuscript.

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