Sedentary behavior and cardiovascular disease risk: mediating mechanisms

Carter, S[1], Hartman, Y[2], Holder, S[1], Thijssen, DH[1,2], Hopkins ND[1].

[1]*Research Institute for Sport and Exercise Science,* Liverpool John Moore's University, Liverpool L3 2ET, UK

[2]Department of Physiology, Radboud University Nijmegen Medical Centre, The Netherlands

Short Title: Sedentary behavior and vascular health

Disclosures: None

Conflict of interest: None

Author for correspondence:

Dr Nicola Hopkins, *Research Institute for Sport and Exercise Science,* Liverpool John Moore's University, Liverpool L3 2ET, UK, email: n.d.hopkins@ljmu.ac.uk, phone: +44(0)151 904 6271, fax: +44 (0)151 904 6284

ABSTRACT

Sedentary behavior (SB) has a strong association with cardiovascular disease (CVD) risk which may be independent of physical activity. To date, the mechanism(s) which mediate this relationship are poorly understood. We hypothesize that SB modifies key hemodynamic, inflammatory and metabolic processes resulting in impaired arterial health. Subsequently, these vascular impairments directly and indirectly contribute to the development of CVD.

SUMMARY

Sedentary behavior modulates key stimuli that impair arterial health, directly and indirectly contributing to the development of cardiovascular disease**.**

KEY POINTS

- Sedentary behavior (SB: i.e. sitting, lying) is associated with numerous negative health connotation. Previous work has predominantly focused on the relationship between SB and all-cause mortality and/or metabolic disorders, yet the relationship between SB and the development of cardiovascular disease (CVD) has received less attention.
- Recent evidence suggests that the negative effects of SB on markers of vascular health, and to a lesser degree traditional CVD risk factors, are likely responsible for the increased CVD incidence and mortality associated with SB.

- We propose that SB induced down regulation of shear rate and blood flow, as well as alterations to glucose metabolism and inflammatory and oxidative stress pathways are likely to play a key role in the vascular dysfunction associated with SB.

KEY WORDS

Sitting, physical activity, vascular function, shear stress, cardiovascular risk factors

GLOSSARY

PA	Physical activity
CV	Cardiovascular
CVD	Cardiovascular disease
SB	Sedentary behavior
HDL	High-density lipoprotein
SFA	Superficial femoral artery
BP	Blood pressure
BA	Brachial artery
ROS	Reactive oxygen species
PI3K	Phosphatidylinositol 3-kinase
MAPK	Mitogen-activated protein kinase

INTRODUCTION

Physical activity (PA) relates to any bodily movement that is associated with energy expenditure, and covers the entire spectrum from moderate- or vigorous-intensity activities (typically referred to as exercise) to, low-intensity activities (i.e. walking, daily life activities). The benefits of regular engagement in low, moderate, and/or vigorous PA have been apparent for some decades and have been frequently described in relation to primary and secondary prevention of cardiovascular disease (CVD). The importance of PA in the context of CVD risk reduction was first highlighted in a seminal study by Morris *et al.* (26), who observed that bus drivers experienced more than double the amount of CVD incidence than bus conductors. The authors speculated that the additional PA undertaken by the bus conductors reduced the risk for future development of CVD. This hypothesis has since been confirmed repeatedly when examining the contribution of PA to CVD morbidity and mortality, culminating in a recent estimate which suggests that physical inactivity, defined as not reaching global PA guidelines, causes 6% of the global CVD burden and has overtaken smoking as the primary cause of all-cause mortality (21).

Evidence from the past decade indicate that PA may not be the only mediator of health and development of non-communicable diseases. Recent studies have provided evidence that sedentary behavior (SB: i.e. sitting, lying) is associated with negative health connotations (8). More specifically, these studies report an association between prolonged periods of SB and all-cause morbidity and mortality, which cannot be simply explained by differences in engagement in low-, moderate- or vigorous-intensity PA (5). Similarly, studies have demonstrated a comparable relationship between SB and development of metabolic disease

(e.g. obesity, metabolic syndrome and type 2 diabetes mellitus). Consequently, SB has emerged as a critical mediator of health in its own right. Due to advances in entertainment and transport technologies, as well as the increased reliance on workplace technology and office work, SB is now the predominant behavior for a large amount of the population in developed countries (28), SB is therefore a clinically relevant target when changing lifestyle behavior.

Previous work has predominantly focused on the relationship between SB and all-cause or CVD mortality and/or metabolic disorders. In this review however, we will focus on the relationship between SB and the development of CVD. More specifically, we aim to synthesize, for the first time, scientific work which examines the relationship between SB and development of CVD and cardiovascular (CV) risk factors, and present an overview of studies that explore the mechanism(s) that underlie this relationship. To meet these aims, this review will *i*) assess whether the negative CV effects are borne from the removal of PA, *ii*) investigate the relationship between SB and CVD risk factors, *iii*) describe the effects of (acute and chronic) uninterrupted sitting on CV health, *iv*) discuss potential mechanisms which mediate the relationship between SB and CVD risk, and *v*) provide recommendations for future directions.

1. Is sedentary behavior simply the removal of activity?

The Australian Diabetes, Obesity and Lifestyle study (AusDiab), a population-based longitudinal study, examined the relationship between SB and CVD development with self-reported television (TV) viewing time as a surrogate marker for SB (8). The authors found that

prolonged periods (≥ 4 hours per day) of TV viewing time was associated with increased risk of CVD mortality, and each additional hour of viewing time was associated with an increased CVD mortality risk of 18%. However, due to limitations associated with correlation analysis, and the confounding relationship between TV viewing and caloric intake, direct independent inferences could not be made. Subsequent studies, however, have provided additional insight into the association between SB and CVD, with a recent meta-analysis of 14 studies confirming that high SB was associated with increased CVD incidence (Hazard Ratio (HR), 1.143 [CI, 1.002 to 1.729]) and mortality (HR, 1.179 [CI, 1.106 to 1.257])(5).

One possible explanation for the associations between SB and CVD risk is that this relationship is merely due to a lack of PA rather than presence of SB. However, the evidence suggests the contrary, in that SB is related to CVD risk independent of engagement in low-, moderate- and/or vigorous-intensity exercise or physical activity (11). For example, in adults meeting weekly PA guidelines, dose-response associations are still observed between television viewing time and cardiometabolic risk factors (e.g. waist circumference, systolic BP, and 2-hour plasma glucose) (12). Moreover, individuals participating in >7 hours/week of moderate-to-vigorous PA, yet also accruing >7 hours daily television watching, present a 2-fold greater risk of CV mortality compared to those engaging in 7 hours/week of moderate-to-vigorous PA and only 1 hour daily television viewing time (23). Furthermore, a recent systematic review and meta-analysis of studies assessing all forms of SB, found that SB was independently associated with increased risk for CVD incidence and mortality regardless of PA (5). Nonetheless, the risk of mortality associated with sitting 10 h/day may be modulated by PA, since the risk for mortality in the sedentary group was 52% higher than those who sit for 1 h/day but were inactive individuals, whereas this risk was reduced to 34% in active individuals

(7). Therefore, these data suggest that the amount of SB is directly (and positively) related to development of CVD, and largely independent from engagement in moderate-to-vigorous PA in the general population.

2. Is sedentary behavior related to cardiovascular risk factors?

The assertion of an independent relationship between SB and CVD mortality and morbidity raises the question of what mechanisms contribute to this observation. One potential, and somewhat obvious explanation relates to the impact of SB on traditional CVD risk factors. In populations of healthy volunteers, studies have provided some evidence for a relationship between SB and traditional CV risk factors. For example, Stamatakis *et al*. observed associations between SB and traditional CV risk (e.g. BMI, waist circumference, BP, HDL cholesterol) in a population of 5,948 healthy, middle aged participants (36). In another cohort of healthy young adults (n=2,328), sitting time was positively and independently associated with resting heart rate and adiposity, and was negatively associated with cardiorespiratory fitness (15). Generally, there is little scientific evidence in healthy populations that SB is related to total cholesterol or levels of low-density lipoproteins (6). However, studies have identified a positive association between SB and high-density lipoprotein (HDL) and triglyceride levels in asymptomatic groups (2, 6, 35), which is largely independent from levels of PA (2, 35).

Relationships between SB and CV risk factors are also apparent in populations with CV risk and/or disease. For example, more time in SB is associated with higher BP ($r=0.56$, $P<0.01$) in hypertensive patients (9). In severely obese subjects, SB shows a positive, independent

relationship with systolic BP, with every additional hour of sitting associated with a 14% higher risk of developing hypertension (17). Beunza *et al.* (4) followed up on these observations in a prospective, dynamic cohort study, which assessed the incidence of hypertension in 6,742 healthy university graduates across a 40-month period. They found that the most sedentary subjects had a 48% increased risk of development of hypertension compared to their non-sedentary peers, (HR 1.48; 95% confidence interval, 1.01 to 2.18, P for trend =0.03) (4), an effect independent of PA levels. Whilst this area of research shows promise, this is very little data available, the role of SB in CV risk factor development in high risk populations therefore warrants further investigation.

In addition to traditional CV risk factors, some recent studies have explored the possible relationship between SB and direct measures of artery health. This is relevant since modulation of traditional CV risk factors alone cannot explain the benefits of PA. The cardioprotective benefits of PA that cannot be explained through traditional risk factors, i.e. the *risk factor gap*, may relate to the effects of PA directly on vascular health (Figure 1). Similarly, SB may also affect CV risk through direct effects on the vasculature. In a cross-sectional analysis of 945 men, after adjusting for covariates (systolic BP and BMI), each additional 30-min of sedentary time was associated with an Odds Ratio of 1.19 (95% CI 1.07, 1.33) for a low ankle-brachial index (i.e. < 0.9) (30). In another cross sectional study it was observed that weekend SB was positively associated with arterial stiffness (males $r=0.11$ $p<0.01$, females $r=0.08$, $p<0.05$), even after adjustment for vigorous PA, resting heart rate, adiposity and metabolic syndrome (15). In addition, in a healthy sample of 614 middle-aged adults, the ratio between sedentary time and time in light intensity PA was positively

associated with carotid intima media thickness (r=0.19, P<0.05) (18). Taken together, these data provide preliminary evidence that SB is related to impaired vascular function and structure, although more work is required to truly understand this complex relationship and assess whether these effects are independent of CV risk factors. This is highly relevant since, together with the impact of SB on traditional risk factors, these effects may contribute to the increased risk for CVD associated with increased SB in both healthy as well as symptomatic populations.

3. Do acute periods of sitting impair cardiovascular health?

To better understand the long-term effect of sitting, recent research has explored the impact of short periods (3-6 h) of sitting on CV outcomes (Table 1). During a 3-hour period of uninterrupted sitting, Padilla *et al.* (29) observed a significant increase in systolic and diastolic BP, evident following 1 hour of sitting and accompanied by a simultaneous decline in popliteal blood flow and shear rate. More recently, Thosar *et al.* (37) observed a decrease in superficial femoral artery (SFA) endothelial function from baseline levels (baseline: 4.72±3.78%, 1hr: 0.52±0.85%, 2hr: 1.66±1.11%, 3hr: 2.2±2.15; p<0.05), and a concomitant reduction in antegrade and mean shear rate in response to a 3-hour period of sitting. This work suggests that lower limb endothelial function is impaired after a period of uninterrupted sitting. In contrast, 3-hours of uninterrupted sitting did not affect brachial artery (BA) endothelial function or mean shear. Importantly, upper limb movement was uncontrolled in this study, whilst previous work on lower limb conduit artery endothelial function and shear rate prevented lower limb movement. Restricting bodily movement therefore appears key for the

down regulation of endothelial function, with even small movements being able to maintain blood flow and endothelial function (39).

The hypothesis that small amounts of movement prevent impairment in CV health associated with prolonged sitting has been explored in more detail in recent studies. Larsen *et al.* observed significantly lower post test systolic and diastolic BPs when 7 hours of sitting was interrupted with self-selected, Borg Scale determined, light- or moderate-intensity 2 minute walking breaks every 20 minutes (SBP: sitting: 123±1 mmHg: light: 120±1 mmHg, P=0.002; moderate: 121±1 mmHg, P=0.02, DBP: sitting: 79±1 mmHg light: 76±1 mmHg, P=0.006; moderate: 77±1 mmHg, P=0.03) (20). When data was stratified according to baseline BP, the largest effects of physical activity breaks on BP were found in those with increased BP *a priori*. These observations suggest that targeting SB may be especially relevant in those with higher CV risk. The impact of regular activity breaks from sitting has also been assessed on triglyceride and lipid levels (5 hours to 3 days). Perhaps unsurprisingly given the duration normally required to alter these parameters, no effect of sitting was observed on triglycerides and lipids (3, 19, 31). Possibly, longer intervention periods are required to impact lipid parameters. Given the limited data on this topic, further research is warranted to better understand the acute effects of breaking up sitting using physical activity on CV risk factors.

The effects of prolonged, uninterrupted sitting and PA breaks have also been explored in relation to measures of endothelial function. In healthy, non-obese men, 5 minutes of light intensity treadmill walking (2 miles.hr^{-1}) every 60 minutes prevented the decline in flow-

mediated dilation and shear rate in SFA observed following 3-hours of uninterrupted sitting (37). In line with these observations, others observed similar beneficial effects of regular activity breaks on SFA flow-mediated dilation in a cohort of healthy young girls (24). This study demonstrated that the reduction in SFA endothelial function associated with 3-hours of prolonged sitting (mean difference 2.2% flow-mediated dilatation; 95% CI=0.60–2.94%, P<0.001) was offset when participants undertook hourly moderate intensity cycling for 10-min. Together, these data support the hypothesis that regular PA breaks, which intermittently increase lower limb muscle activity level, prevent the decline in resting flow and endothelial function associated with prolonged, uninterrupted sitting. More recently, studies have had some success in preventing sitting induced endothelial dysfunction using alternative physical activity interventions such as fidgeting (25), which was found to prevent the decline in blood flow and shear rate observed during prolonged, uninterrupted sitting. These data suggest that sitting-induced endothelial dysfunction can be offset via interventions which promote (low-intensity) physical activity.

4. Does chronic sitting impair cardiovascular health?

To our knowledge, few studies have directly examined the consequences of longer term, acute exposure (>1 day) to SB on CV risk factors. Lyden and colleagues investigated the effects of seven days increased sitting time and reduced breaks from sitting on lipids and markers of insulin resistance in 10 healthy young subjects. Compared to baseline, seven days of SB caused no change in fasting plasma lipids, waist circumference and BMI. Nonetheless, 2-h plasma insulin and area-under-the-curve, as measured by oral glucose tolerance test, were significantly increased after the 7-day period of increased SB, indicative of the ability of SB to

cause insulin resistance within 1 week (22). Another study explored the impact of a 3-day intervention of either 7 hours per day of uninterrupted sitting, or 7 hours of sitting with 2 minute light intensity walks every 20 minutes (19). Significantly lower glucose and insulin area-under-the-curve, as measured by mixed meal tolerance test, was found following only 3-days of uninterrupted sitting to compared to the break condition. In line with previous findings, triglycerides did not differ between uninterrupted sitting and intervention. Therefore, studies on the immediate (3-6 h) and short-term (3-7 days) effects of SB suggest the presence of significant impairment in measures of insulin resistance, whilst no such changes were present in lipid levels.

A likely explanation for the absence of an effect of (short-term) SB on lipids may relate to the duration of the intervention. In line with work related to exercise training, longer-term interventions may be required to alter lipid levels. Indeed, a study in which office workers reduced sitting time by 137 minutes over a 3-month intervention period, showed positive effects on HDL cholesterol (1). Similarly, in a study by Graves *et al*., self-selected use of standing work stations resulted in a mean reduction in sitting time of 90 minutes per day over an 8-week intervention period (10). Consequently, a significant reduction in total cholesterol level was observed, supporting the idea that prolonged periods of physical (in)activity are required to alter lipid levels. Additionally, this is the only study to our knowledge, to have examined the endothelial response to long-term reductions in sitting time. Despite non-significant effects, the study also performed inferential statistics which indicated 'potentially clinically meaningful' improvements in BA endothelial function as well as 'possible benefits' in the reduction in diastolic BP after the 8-week intervention that reduced sitting time with ~90-minutes per day. In conclusion, short- and long-term periods of SB are able to alter

peripheral blood flow and artery endothelial function, as well BP. Future work is required to better understand these effects, especially since these impacts may differ between groups or between different interventions to alter SB.

5. What are the mechanism(s) underlying SB-induced changes in vascular function?

Recent experimental evidence suggests that changes in CV risk factors, but also impairments to vascular health, likely contribute to the potentially detrimental effects of prolonged, uninterrupted SB on CVD (Figure 2). Here we will focus on understanding the mechanisms underlying the impairment in vascular health.

Hemodynamic stimuli

Previous work has revealed a central role for hemodynamic stimuli, such as shear stress, in mediating functional and structural changes in vascular health (16). Similarly, prolonged, uninterrupted sitting is associated with changes in shear that can mediate vascular dysfunction. Early work, which used prolonged sitting as a model to increase hydrostatic pressure on the lower limbs, observed that sitting for three hours reduced mean, minimum and maximum shear rate in the popliteal artery (29). Indirect evidence for shear stress to mediate changes in endothelial function with SB is provided by Restaino and colleagues (32). They found 3-4 hours of sitting reduced blood flow and shear rate in lower limb conduit arteries, but not in the brachial arteries. These observations were associated with a decline in lower limb artery endothelial function, but not BA endothelial function. Another study examining lower and upper limb blood flow and endothelial function using an uninterrupted sitting period of 3-h (39) noted reductions in both popliteal and brachial shear, but only

popliteal endothelial function. The BA may therefore exhibit greater resilience to shear rate reductions or longer periods are required to successfully affect perfusion and/or vascular health (32).

Whilst these observations provide some indirect evidence for the role of shear, more recent work directly explored the hypothesis that changes in shear mediate the decrease in endothelial function during prolonged, uninterrupted sitting. In healthy young men, popliteal artery endothelial dysfunction induced by three hours of uninterrupted sitting was successfully attenuated by manipulating popliteal perfusion by local heating (33) or by small fidgeting leg movements (25). Both interventions successfully prevented the reduction in mean shear associated with prolonged, uninterrupted sitting. Consequently, both studies maintained or increased mean shear in the lower limbs from baseline levels, either through metabolic flow (i.e. PA related (pre-fidgeting: 33.7±2.6sec^{-1} to immediately post-fidgeting: 222.7±28.3sec^{-1}; $P<0.001$)) or non-metabolic flow (i.e. induced by heat (pre-sit, 38.9 ± 3.4 sec^{-1}; and 3-h sit, 63.9 ± 16.9 sec^{-1}; $P > 0.05$), which successfully prevented the reduction in popliteal artery endothelial function.

In addition to reductions in mean shear rate, the patterns of shear may be equally important. Shear patterns have a key role in maintaining vascular function; antegrade shear stress preserves or enhances endothelial function by activating nitric oxide production; whilst low and oscillatory shear stress can promote atherosclerosis, inflammation and increased oxidative stress (16). Antegrade shear is reduced in the SFA and BA during three hours of uninterrupted sitting, whilst oscillatory shear is increased in the BA (39). Interestingly, changes in the shear pattern of both vessels occurred over distinct time courses. The

reduction in femoral artery antegrade shear was evident after only one hour of sitting, which coincided with the reduction in SFA endothelial function. In contrast however, changes in BA antegrade and oscillatory shear were observed following three hours of uninterrupted sitting. These data indicate that over a relatively short time scale (1 hr), uninterrupted sitting elicits negative effects on antegrade and oscillatory shear, and consequently endothelial function in the lower limbs. Whilst the negative SB effects on shear patterns in the upper limb occur over a longer period of time (3hrs) and are not accompanied by endothelial reductions (39). Studies of a longer duration are needed to fully examine the effects of sitting induced alterations in shear pattern on endothelial function.

The underlying mechanisms that contribute to the changes in shear are currently unknown. One potential mechanism may relate to the exposure to prolonged gravitational forces, which may increase hydrostatic pressure within the lower limbs, causing venous pooling and subsequent reductions in blood flow and shear stress (32). Prolonged sitting leads to increased calf circumference (32), calf pooling and decreased thigh blood flow (40). Additionally, increased muscle sympathetic nerve activity and changes in blood viscosity may also contribute to altered shear rates and endothelial dysfunction (32). Insight into the pathways that underlie the dysfunctional vascular environment in response to sitting is also limited. Previous work provides strong evidence that lower shear rates decrease nitric oxide availability and increase production of vasoconstrictors such as endothelin-1, whilst lower shear rate is also conducive to the expression of atherogenic genes and inhibition of anti-atherogenic genes (40). Compared to standing, sitting elicits changes in the angles of the major arteries which may increase turbulent flow and shear patterns known to augment the

atherosclerotic process (40). These factors may all, in part, contribute to the strong link between prolonged, uninterrupted sitting and impaired vascular health.

Inflammation and Reactive Oxygen Species

There is a strong association between elevated inflammatory markers and impaired vascular function. Inflammatory markers are also associated with reduced nitric oxide availability, CVD incidence and risk prediction (13, 41). Previous work found that higher self-reported sitting is associated with higher levels of adipokines and low-grade inflammation, an observation that was independent of PA levels (14). Cross-sectional and prospective studies have also shown that longer sitting time is associated with higher levels of C-reactive protein, a marker of systemic inflammation (13, 14). However, this association between C-reactive protein and SB was attenuated or lost after controlling for BMI or waist circumference, suggesting that adiposity levels may mediate this relationship (13, 14). Despite the presence of some observational evidence that sitting is associated with the presence of markers of (low-grade) inflammation, no study has directly examined the impact of SB on these markers and/or linked these changes in markers of inflammation to changes in endothelial function.

Inflammatory cytokines also activate vascular production of reactive oxygen species (ROS) (41), which may further explain the association between SB and CVD risk. Production of ROS is regarded as an important component in the pathogenesis of CVD, particularly due to the production of superoxide which is associated with impairments to endothelial function and hypertension (41). Interestingly, a recent study examined whether the reduction in SFA endothelial function following three hours of uninterrupted sitting could be prevented by oral administration of Vitamin C, a potent ROS scavenger (38). Whilst the sitting-induced reduction

in SFA endothelial function was successfully prevented by intake of vitamin C, this study did not perform additional testing to confirm that Vitamin C was indeed responsible for a reduction in oxidative stress. These initial findings support further work to focus on a potential role for ROS to contribute to the impact of prolonged sitting on vascular health.

Metabolic markers

The link between SB and metabolic health has been well documented, with experimental research demonstrating detrimental changes in blood insulin and glucose levels as a result of prolonged, uninterrupted sitting. Insulin resistance is associated with endothelial dysfunction due to an imbalance between the phosphatidylinositol 3-kinase (PI3K)-dependent and mitogen-activated protein kinase (MAPK)-dependent signaling pathways (27). In an insulin resistant state, PI3K signaling is reduced leading to decreased nitric oxide availability, whilst MAPK signaling is unaffected, leading to greater endothlin-1 production, endothelial cell apoptosis and inflammation (27). Furthermore, sedentary time results in longer periods of postprandial hyperglycemia (27), which may have a mechanistic contribution, as acute and prolonged periods of hyperglycemia are also known to impair endothelial function. The effects of hyperglycaemia occur via several mechanism including increased ROS production, increased advanced glycation end products formation, and the activation of protein kinase C, which ultimately results in heightened oxidative stress, apoptosis and increased vascular permeability (34). Therefore, the detrimental effect of sitting on CV health may, at least in part, be the result of metabolic dysfunction and its subsequent effects on the vasculature.

6. Conclusion and future directions

A significant body of evidence indicates that prolonged sitting is associated with increased risk for developing CVD and that this association between SB and CVD cannot simply be explained by the absence of moderate to vigorous PA. Research provides strong evidence that this link is, at least partially, due to sitting-induced alterations to traditional CV risk factors, including glucose tolerance, BP and lipid profile (via HDL) as well as impairment in vascular health mediated by reductions in mean and antegrade blood flow and shear rate. Recent work also highlights a potential role for increased ROS production, presence of low-grade inflammation and metabolic impairment to contribute to sitting induced impaired vascular function. However, this field is in its infancy and several important questions needs to be answered to better understand the impact of SB on CV health.

Lab studies indicate that regularly interrupting sitting appears to be more important than the total duration of sitting to prevent the effects on the CV system, however epidemiological evidence for this is currently lacking. Furthermore, relatively little is known, both from epidemiological and (pre)clinical work, whether the impact of targeting SB is equally relevant in healthy, physically active subjects *versus* physically inactive subjects with or without CV disease and/or risk. Better understanding in this area would highlight which population would potentially benefit most. To further understand potential mechanisms underlying sitting induced vascular dysfunction and increased CVD risk, studies should explore blood-borne markers such as circulating endothelial progenitor cells and whether (long-term) SB may contribute to changes in measures of vascular structure. Furthermore, research has largely focused on the influence of SB on conduit arteries, other vascular beds, including the coronary, peripheral resistance and cerebrovascular beds should be explored.

To date, the direct influence of SB on CVD (risk factors) has only been studied after a short period of altered SB. This highlights the need for well-designed and properly powered studies that examine the impact of longer-term follow-up on both the impact of SB, as well as interventions that counteract these detrimental effects. This work is required to better understand the impact of SB, to enhance the ecological value of research in this area, and importantly to develop specific public health guidelines for daily sitting time.

References

1. Alkhajah TA, Reeves MM, Eakin EG, Winkler EA, Owen N, Healy GN. Sit–Stand workstations: a pilot intervention to reduce office sitting time. *American journal of preventive medicine*. 2012;43(3):298-303.
2. Altenburg TM, Lakerveld J, Bot SD, Nijpels G, Chinapaw MJ. The prospective relationship between sedentary time and cardiometabolic health in adults at increased cardiometabolic risk–the Hoorn Prevention Study. *International Journal of Behavioral Nutrition and Physical Activity*. 2014;11(1):1.
3. Bailey DP, Locke CD. Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J Sci Med Sport*. 2015;18(3):294-8.
4. Beunza JJ, Martínez-González MÁ, Ebrahim S et al. Sedentary Behaviors and the Risk of Incident Hypertension* The SUN Cohort. *American journal of hypertension*. 2007;20(11):1156-62.
5. Biswas A, Oh PI, Faulkner GE et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Annals of Internal Medicine*. 2015;162(2):123-32.
6. Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. *Preventive medicine*. 2015;76:92-102.
7. Chau JY, Grunseit AC, Chey T et al. Daily sitting time and all-cause mortality: a meta-analysis. *PLoS One*. 2013;8(11):e80000.
8. Dunstan DW, Barr EL, Healy GN et al. Television viewing time and mortality: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). *Circulation*. 2010;121(3):384-91.
9. Gerage AM, Benedetti TR, Farah BQ et al. Sedentary Behavior and Light Physical Activity Are Associated with Brachial and Central Blood Pressure in Hypertensive Patients. *PLoS One*. 2015;10(12):e0146078.
10. Graves L, Murphy R, Shepherd SO, Cabot J, Hopkins ND. Evaluation of sit-stand workstations in an office setting: a randomised controlled trial. *BMC public health*. 2015;15(1):1.
11. Hamilton MT, Healy GN, Dunstan DW, Zderic TW, Owen N. Too Little Exercise and Too Much Sitting: Inactivity Physiology and the Need for New Recommendations on Sedentary Behavior. *Curr Cardiovasc Risk Rep*. 2008;2(4):292-8.
12. Healy GN, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N. Television time and continuous metabolic risk in physically active adults. *Medicine and science in sports and exercise*. 2008;40(4):639-45.
13. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J*. 2011;32(5):590-7.
14. Howard BJ, Balkau B, Thorp AA et al. Associations of overall sitting time and TV viewing time with fibrinogen and C reactive protein: the AusDiab study. *British journal of sports medicine*. 2015;49(4):255-8.
15. Huynh QL, Blizzard CL, Sharman JE, Magnussen CG, Dwyer T, Venn AJ. The cross-sectional association of sitting time with carotid artery stiffness in young adults. *BMJ open*. 2014;4(3):e004384.
16. Johnson BD, Mather KJ, Wallace JP. Mechanotransduction of shear in the endothelium: basic studies and clinical implications. *Vasc Med*. 2011;16(5):365-77.
17. King WC, Chen JY, Courcoulas AP et al. Objectively-measured sedentary time and cardiometabolic health in adults with severe obesity. *Preventive medicine*. 2016;84:12-8.
18. Kozàkovà M, Palombo C, Morizzo C et al. Effect of sedentary behaviour and vigorous physical activity on segment-specific carotid wall thickness and its progression in a healthy population. *European heart journal*. 2010;31(12):1511-9.

19. Larsen RN, Kingwell BA, Robinson C et al. Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults. *Clinical science*. 2015;129(2):117-27.
20. Larsen RN, Kingwell BA, Sethi P, Cerin E, Owen N, Dunstan DW. Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults. *Nutr Metab Cardiovasc Dis*. 2014;24(9):976-82.
21. Lee IM, Shiroma EJ, Lobelo F et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet*. 2012;380(9838):219-29.
22. Lyden K, Keadle SK, Staudenmayer J, Braun B, Freedson PS. Discrete features of sedentary behavior impact cardiometabolic risk factors. *Medicine and science in sports and exercise*. 2015;47(5):1079-86.
23. Matthews CE, George SM, Moore SC et al. Amount of time spent in sedentary behaviors and cause-specific mortality in US adults. *The American journal of clinical nutrition*. 2012;95(2):437-45.
24. McManus AM, Ainslie PN, Green DJ, Simair RG, Smith K, Lewis N. Impact of prolonged sitting on vascular function in young girls. *Experimental physiology*. 2015;100(11):1379-87.
25. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, Padilla J. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. *American journal of physiology*. 2016;311(1):H177-82.
26. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical activity of work. *Lancet*. 1953;265(6796):1111-20.
27. Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. *Rev Endocr Metab Disord*. 2013;14(1):5-12.
28. Owen N, Bauman A, Brown W. Too much sitting: a novel and important predictor of chronic disease risk? *British journal of sports medicine*. 2009;43(2):81-3.
29. Padilla J, Sheldon RD, Sitar DM, Newcomer SC. Impact of acute exposure to increased hydrostatic pressure and reduced shear rate on conduit artery endothelial function: a limb-specific response. *American journal of physiology*. 2009;297(3):H1103-8.
30. Parsons TJ, Sartini C, Ellins EA et al. Objectively measured physical activity and sedentary behaviour and ankle brachial index: Cross-sectional and longitudinal associations in older men. *Atherosclerosis*. 2016;247:28-34.
31. Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *The American journal of clinical nutrition*. 2013;98(2):358-66.
32. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. *Experimental physiology*. 2015;100(7):829-38.
33. Restaino RM, Walsh LK, Morishima T et al. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. *American journal of physiology*. 2016;310(5):H648-53.
34. Sena CM, Pereira AM, Seica R. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta*. 2013;1832(12):2216-31.
35. Shuval K, Finley CE, Barlow CE, Gabriel KP, Leonard D, Kohl HW. Sedentary behavior, cardiorespiratory fitness, physical activity, and cardiometabolic risk in men: the cooper center longitudinal study. In: *Proceedings of the Mayo Clinic Proceedings*. 2014. p. 1052-62.
36. Stamatakis E, Hamer M, Tilling K, Lawlor DA. Sedentary time in relation to cardio-metabolic risk factors: differential associations for self-report vs accelerometry in working age adults. *Int J Epidemiol*. 2012;41(5):1328-37.

37. Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of prolonged sitting and breaks in sitting time on endothelial function. *Medicine and science in sports and exercise*. 2015;47(4):843-9.
38. Thosar SS, Bielko SL, Wiggins CC, Klaunig JE, Mather KJ, Wallace JP. Antioxidant vitamin C prevents decline in endothelial function during sitting. *Med Sci Monit*. 2015;21:1015-21.
39. Thosar SS, Bielko SL, Wiggins CC, Wallace JP. Differences in brachial and femoral artery responses to prolonged sitting. *Cardiovasc Ultrasound*. 2014;12:50.
40. Thosar SS, Johnson BD, Johnston JD, Wallace JP. Sitting and endothelial dysfunction: the role of shear stress. *Med Sci Monit*. 2012;18(12):RA173-80.
41. Zhang N, Andresen BT, Zhang C. Inflammation and reactive oxygen species in cardiovascular disease. *World J Cardiol*. 2010;2(12):408-10.

1

FIGURE LEGENDS

Figure 1: Schematic depicting the effect of SB on CVD risk factors: A) Vascular structure and function: stiffness and intima-media thickness increase; endothelial function decreases, B) Body mass index increases, C) Cardiorespiratory fitness decreases D) Blood pressure increases E) Insulin resistance increases, F) Blood lipids increase

Figure 2: Summary of the potential mechanisms underlying sitting-induced cardiovascular disease risk. a) Represents an artery during walking whilst b) represents an artery following a period of sedentary behaviour, whereby blood flow and shear stress are reduced, attenuating nitric oxide (NO) and increasing endothelin (ET-1) production, subsequently leading to vascular dysfunction. Additionally, insulin resistance, inflammation and reactive oxygen species (ROS) production may further augment this vascular dysfunction**.**

TABLE LEGEND

Table 1 Summary of studies examining the cardiovascular effects of sitting interventions

Table 1 Summary of studies examining the cardiovascular effects of sitting interventions

First author	N	Follow-up	Population	Intervention	Outcome variable	Conclusion
Peddie (31)	70	9-h	Adults	1.40-min breaks every 30 min	Triglycerides, glucose, insulin	No difference in triglyceride levels between activity breaks and prolonged sitting
Bailey (3)	10	5-h	Adults	2-min breaks every 20 min	Blood pressure, lipids glucose	No difference in BP and lipid levels between activity breaks and prolonged sitting
Larsen (19)	19	3-days	Overweight	2-min breaks every 20 min	Triglycerides, glucose, insulin	No difference in triglyceride levels after 1 and 3 days between interrupted and uninterrupted sitting.
Larsen (20)	19	7-h	Overweight	2-min breaks every 20 min	Blood pressure, heart rate	Lower SBP and DBP compared to prolonged sitting, no difference in mean arterial pressure or heart rate
Thosar (37)	12	3-h	Healthy men	5-min breaks every hour	SFA FMD, shear rate	Decline in FMD was prevented compared to prolonged sitting
Restaino (33)	10	3-h	Healthy men	Increased shear (using heat) during sitting	SFA FMD, shear rate	Impaired FMD during prolonged sitting was prevented by increasing shear
Morishima (25)	11	3-h	Healthy adults	1-min fidgeting breaks every 4 min.	SFA FMD	Fidgeting prevented a decline in shear rate and flow and improved FMD.
Graves (10)	47	8-wk	Adults	Sit-stand work station	BA FMD, BP, glucose, blood lipids	Improved cholesterol, possible benefits in blood pressure and FMD

FMD- Flow mediated dilation used to assess endothelial function, SFA – Superficial femoral artery, BA – Brachial artery.