



LJMU Research Online

Carter, SE, Hartman, Y, Holder, S, Thijssen, DHJ and Hopkins, ND

Sedentary Behavior and Cardiovascular Disease Risk: Mediating Mechanisms.

<http://researchonline.ljmu.ac.uk/id/eprint/6293/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Carter, SE, Hartman, Y, Holder, S, Thijssen, DHJ and Hopkins, ND (2017)
Sedentary Behavior and Cardiovascular Disease Risk: Mediating
Mechanisms. Exercise and Sport Sciences Reviews, 45 (2). pp. 80-86. ISSN
1538-3008**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Sedentary behavior and cardiovascular disease risk: mediating mechanisms

Carter, S¹, Hartman, Y², Holder, S¹, Thijssen, DH^{1,2}, Hopkins ND¹.

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

²Department 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*

1 **ABSTRACT**

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

7

8 **SUMMARY**

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

11

12 **KEY POINTS**

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

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

4

5 **KEY WORDS**

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

7 **GLOSSARY**

8	PA	Physical activity
9	CV	Cardiovascular
10	CVD	Cardiovascular disease
11	SB	Sedentary behavior
12	HDL	High-density lipoprotein
13	SFA	Superficial femoral artery
14	BP	Blood pressure
15	BA	Brachial artery
16	ROS	Reactive oxygen species
17	PI3K	Phosphatidylinositol 3-kinase
18	MAPK	Mitogen-activated protein kinase

19

20

21

22

1 **INTRODUCTION**

2 Physical activity (PA) relates to any bodily movement that is associated with energy
3 expenditure, and covers the entire spectrum from moderate- or vigorous-intensity activities
4 (typically referred to as exercise) to, low-intensity activities (i.e. walking, daily life activities).

5 The benefits of regular engagement in low, moderate, and/or vigorous PA have been
6 apparent for some decades and have been frequently described in relation to primary and
7 secondary prevention of cardiovascular disease (CVD). The importance of PA in the context
8 of CVD risk reduction was first highlighted in a seminal study by Morris *et al.* (26), who
9 observed that bus drivers experienced more than double the amount of CVD incidence than
10 bus conductors. The authors speculated that the additional PA undertaken by the bus
11 conductors reduced the risk for future development of CVD. This hypothesis has since been
12 confirmed repeatedly when examining the contribution of PA to CVD morbidity and mortality,
13 culminating in a recent estimate which suggests that physical inactivity, defined as not
14 reaching global PA guidelines, causes 6% of the global CVD burden and has overtaken smoking
15 as the primary cause of all-cause mortality (21).

16

17 Evidence from the past decade indicate that PA may not be the only mediator of health and
18 development of non-communicable diseases. Recent studies have provided evidence that
19 sedentary behavior (SB: i.e. sitting, lying) is associated with negative health connotations (8).

20 More specifically, these studies report an association between prolonged periods of SB and
21 all-cause morbidity and mortality, which cannot be simply explained by differences in
22 engagement in low-, moderate- or vigorous-intensity PA (5). Similarly, studies have
23 demonstrated a comparable relationship between SB and development of metabolic disease

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

7

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

18

19 **1. Is sedentary behavior simply the removal of activity?**

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

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

9

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

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

4 5 **2. Is sedentary behavior related to cardiovascular risk factors?**

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

20
21 Relationships between SB and CV risk factors are also apparent in populations with CV risk
22 and/or disease. For example, more time in SB is associated with higher BP ($r=0.56$, $P<0.01$) in
23 hypertensive patients (9). In severely obese subjects, SB shows a positive, independent

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

10

11 In addition to traditional CV risk factors, some recent studies have explored the possible
12 relationship between SB and direct measures of artery health. This is relevant since
13 modulation of traditional CV risk factors alone cannot explain the benefits of PA. The
14 cardioprotective benefits of PA that cannot be explained through traditional risk factors, i.e.
15 the *risk factor gap*, may relate to the effects of PA directly on vascular health (Figure 1).
16 Similarly, SB may also affect CV risk through direct effects on the vasculature. In a cross-
17 sectional analysis of 945 men, after adjusting for covariates (systolic BP and BMI), each
18 additional 30-min of sedentary time was associated with an Odds Ratio of 1.19 (95% CI 1.07,
19 1.33) for a low ankle-brachial index (i.e. < 0.9) (30). In another cross sectional study it was
20 observed that weekend SB was positively associated with arterial stiffness (males $r=0.11$
21 $p<0.01$, females $r=0.08$, $p<0.05$), even after adjustment for vigorous PA, resting heart rate,
22 adiposity and metabolic syndrome (15). In addition, in a healthy sample of 614 middle-aged
23 adults, the ratio between sedentary time and time in light intensity PA was positively

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

8

9 **3. Do acute periods of sitting impair cardiovascular health?**

10 To better understand the long-term effect of sitting, recent research has explored the impact
11 of short periods (3-6 h) of sitting on CV outcomes (Table 1). During a 3-hour period of
12 uninterrupted sitting, Padilla *et al.* (29) observed a significant increase in systolic and diastolic
13 BP, evident following 1 hour of sitting and accompanied by a simultaneous decline in popliteal
14 blood flow and shear rate. More recently, Thosar *et al.* (37) observed a decrease in superficial
15 femoral artery (SFA) endothelial function from baseline levels (baseline: $4.72\pm3.78\%$, 1hr:
16 $0.52\pm0.85\%$, 2hr: $1.66\pm1.11\%$, 3hr: 2.2 ± 2.15 ; $p<0.05$), and a concomitant reduction in
17 antegrade and mean shear rate in response to a 3-hour period of sitting. This work suggests
18 that lower limb endothelial function is impaired after a period of uninterrupted sitting. In
19 contrast, 3-hours of uninterrupted sitting did not affect brachial artery (BA) endothelial
20 function or mean shear. Importantly, upper limb movement was uncontrolled in this study,
21 whilst previous work on lower limb conduit artery endothelial function and shear rate
22 prevented lower limb movement. Restricting bodily movement therefore appears key for the

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

3

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

19

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

1 mediated dilation and shear rate in SFA observed following 3-hours of uninterrupted sitting
2 (37). In line with these observations, others observed similar beneficial effects of regular
3 activity breaks on SFA flow-mediated dilation in a cohort of healthy young girls (24). This study
4 demonstrated that the reduction in SFA endothelial function associated with 3-hours of
5 prolonged sitting (mean difference 2.2% flow-mediated dilatation; 95% CI=0.60–2.94%,
6 $P<0.001$) was offset when participants undertook hourly moderate intensity cycling for 10-
7 min. Together, these data support the hypothesis that regular PA breaks, which intermittently
8 increase lower limb muscle activity level, prevent the decline in resting flow and endothelial
9 function associated with prolonged, uninterrupted sitting. More recently, studies have had
10 some success in preventing sitting induced endothelial dysfunction using alternative physical
11 activity interventions such as fidgeting (25), which was found to prevent the decline in blood
12 flow and shear rate observed during prolonged, uninterrupted sitting. These data suggest that
13 sitting-induced endothelial dysfunction can be offset via interventions which promote (low-
14 intensity) physical activity.

15

16 **4. Does chronic sitting impair cardiovascular health?**

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

1 cause insulin resistance within 1 week (22). Another study explored the impact of a 3-day
2 intervention of either 7 hours per day of uninterrupted sitting, or 7 hours of sitting with 2
3 minute light intensity walks every 20 minutes (19). Significantly lower glucose and insulin
4 area-under-the-curve, as measured by mixed meal tolerance test, was found following only
5 3-days of uninterrupted sitting to compared to the break condition. In line with previous
6 findings, triglycerides did not differ between uninterrupted sitting and intervention.
7 Therefore, studies on the immediate (3-6 h) and short-term (3-7 days) effects of SB suggest
8 the presence of significant impairment in measures of insulin resistance, whilst no such
9 changes were present in lipid levels.

10

11 A likely explanation for the absence of an effect of (short-term) SB on lipids may relate to the
12 duration of the intervention. In line with work related to exercise training, longer-term
13 interventions may be required to alter lipid levels. Indeed, a study in which office workers
14 reduced sitting time by 137 minutes over a 3-month intervention period, showed positive
15 effects on HDL cholesterol (1). Similarly, in a study by Graves *et al.*, self-selected use of
16 standing work stations resulted in a mean reduction in sitting time of 90 minutes per day over
17 an 8-week intervention period (10). Consequently, a significant reduction in total cholesterol
18 level was observed, supporting the idea that prolonged periods of physical (in)activity are
19 required to alter lipid levels. Additionally, this is the only study to our knowledge, to have
20 examined the endothelial response to long-term reductions in sitting time. Despite non-
21 significant effects, the study also performed inferential statistics which indicated 'potentially
22 clinically meaningful' improvements in BA endothelial function as well as 'possible benefits'
23 in the reduction in diastolic BP after the 8-week intervention that reduced sitting time with
24 ~90-minutes per day. In conclusion, short- and long-term periods of SB are able to alter

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

4

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

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

10

11 *Hemodynamic stimuli*

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

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

4
5 Whilst these observations provide some indirect evidence for the role of shear, more recent
6 work directly explored the hypothesis that changes in shear mediate the decrease in
7 endothelial function during prolonged, uninterrupted sitting. In healthy young men, popliteal
8 artery endothelial dysfunction induced by three hours of uninterrupted sitting was
9 successfully attenuated by manipulating popliteal perfusion by local heating (33) or by small
10 fidgeting leg movements (25). Both interventions successfully prevented the reduction in
11 mean shear associated with prolonged, uninterrupted sitting. Consequently, both studies
12 maintained or increased mean shear in the lower limbs from baseline levels, either through
13 metabolic flow (i.e. PA related (pre-fidgeting: $33.7 \pm 2.6 \text{sec}^{-1}$ to immediately post-fidgeting:
14 $222.7 \pm 28.3 \text{sec}^{-1}$; $P < 0.001$)) or non-metabolic flow (i.e. induced by heat (pre-sit, 38.9 ± 3.4
15 sec^{-1} ; and 3-h sit, $63.9 \pm 16.9 \text{sec}^{-1}$; $P > 0.05$), which successfully prevented the reduction in
16 popliteal artery endothelial function.

17
18 In addition to reductions in mean shear rate, the patterns of shear may be equally important.
19 Shear patterns have a key role in maintaining vascular function; antegrade shear stress
20 preserves or enhances endothelial function by activating nitric oxide production; whilst low
21 and oscillatory shear stress can promote atherosclerosis, inflammation and increased
22 oxidative stress (16). Antegrade shear is reduced in the SFA and BA during three hours of
23 uninterrupted sitting, whilst oscillatory shear is increased in the BA (39). Interestingly,
24 changes in the shear pattern of both vessels occurred over distinct time courses. The

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

10

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

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

3

4 *Inflammation and Reactive Oxygen Species*

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

17

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

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

5

6 *Metabolic markers*

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

22

23 **6. Conclusion and future directions**

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

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

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

7

8

1 References

- 2 1. Alkhajah TA, Reeves MM, Eakin EG, Winkler EA, Owen N, Healy GN. Sit–Stand workstations:
3 a pilot intervention to reduce office sitting time. *American journal of preventive medicine*.
4 2012;43(3):298-303.
- 5 2. Altenburg TM, Lakerveld J, Bot SD, Nijpels G, Chinapaw MJ. The prospective relationship
6 between sedentary time and cardiometabolic health in adults at increased cardiometabolic
7 risk—the Hoorn Prevention Study. *International Journal of Behavioral Nutrition and Physical*
8 *Activity*. 2014;11(1):1.
- 9 3. Bailey DP, Locke CD. Breaking up prolonged sitting with light-intensity walking improves
10 postprandial glycemia, but breaking up sitting with standing does not. *J Sci Med Sport*.
11 2015;18(3):294-8.
- 12 4. Beunza JJ, Martínez-González MÁ, Ebrahim S et al. Sedentary Behaviors and the Risk of
13 Incident Hypertension* The SUN Cohort. *American journal of hypertension*.
14 2007;20(11):1156-62.
- 15 5. Biswas A, Oh PI, Faulkner GE et al. Sedentary time and its association with risk for disease
16 incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis.
17 *Annals of Internal Medicine*. 2015;162(2):123-32.
- 18 6. Brocklebank LA, Falconer CL, Page AS, Perry R, Cooper AR. Accelerometer-measured
19 sedentary time and cardiometabolic biomarkers: A systematic review. *Preventive medicine*.
20 2015;76:92-102.
- 21 7. Chau JY, Grunseit AC, Chey T et al. Daily sitting time and all-cause mortality: a meta-analysis.
22 *PLoS One*. 2013;8(11):e80000.
- 23 8. Dunstan DW, Barr EL, Healy GN et al. Television viewing time and mortality: the Australian
24 Diabetes, Obesity and Lifestyle Study (AusDiab). *Circulation*. 2010;121(3):384-91.
- 25 9. Gerage AM, Benedetti TR, Farah BQ et al. Sedentary Behavior and Light Physical Activity Are
26 Associated with Brachial and Central Blood Pressure in Hypertensive Patients. *PLoS One*.
27 2015;10(12):e0146078.
- 28 10. Graves L, Murphy R, Shepherd SO, Cabot J, Hopkins ND. Evaluation of sit-stand workstations
29 in an office setting: a randomised controlled trial. *BMC public health*. 2015;15(1):1.
- 30 11. Hamilton MT, Healy GN, Dunstan DW, Zderic TW, Owen N. Too Little Exercise and Too Much
31 Sitting: Inactivity Physiology and the Need for New Recommendations on Sedentary
32 Behavior. *Curr Cardiovasc Risk Rep*. 2008;2(4):292-8.
- 33 12. Healy GN, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N. Television time and
34 continuous metabolic risk in physically active adults. *Medicine and science in sports and*
35 *exercise*. 2008;40(4):639-45.
- 36 13. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-
37 metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J*. 2011;32(5):590-7.
- 38 14. Howard BJ, Balkau B, Thorp AA et al. Associations of overall sitting time and TV viewing time
39 with fibrinogen and C reactive protein: the AusDiab study. *British journal of sports medicine*.
40 2015;49(4):255-8.
- 41 15. Huynh QL, Blizzard CL, Sharman JE, Magnussen CG, Dwyer T, Venn AJ. The cross-sectional
42 association of sitting time with carotid artery stiffness in young adults. *BMJ open*.
43 2014;4(3):e004384.
- 44 16. Johnson BD, Mather KJ, Wallace JP. Mechanotransduction of shear in the endothelium: basic
45 studies and clinical implications. *Vasc Med*. 2011;16(5):365-77.
- 46 17. King WC, Chen JY, Courcoulas AP et al. Objectively-measured sedentary time and
47 cardiometabolic health in adults with severe obesity. *Preventive medicine*. 2016;84:12-8.
- 48 18. Kozàková M, Palombo C, Morizzo C et al. Effect of sedentary behaviour and vigorous physical
49 activity on segment-specific carotid wall thickness and its progression in a healthy
50 population. *European heart journal*. 2010;31(12):1511-9.

- 1 19. Larsen RN, Kingwell BA, Robinson C et al. Breaking up of prolonged sitting over three days
2 sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in
3 overweight and obese adults. *Clinical science*. 2015;129(2):117-27.
- 4 20. Larsen RN, Kingwell BA, Sethi P, Cerin E, Owen N, Dunstan DW. Breaking up prolonged sitting
5 reduces resting blood pressure in overweight/obese adults. *Nutr Metab Cardiovasc Dis*.
6 2014;24(9):976-82.
- 7 21. Lee IM, Shiroma EJ, Lobelo F et al. Effect of physical inactivity on major non-communicable
8 diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet*.
9 2012;380(9838):219-29.
- 10 22. Lyden K, Keadle SK, Staudenmayer J, Braun B, Freedson PS. Discrete features of sedentary
11 behavior impact cardiometabolic risk factors. *Medicine and science in sports and exercise*.
12 2015;47(5):1079-86.
- 13 23. Matthews CE, George SM, Moore SC et al. Amount of time spent in sedentary behaviors and
14 cause-specific mortality in US adults. *The American journal of clinical nutrition*.
15 2012;95(2):437-45.
- 16 24. McManus AM, Ainslie PN, Green DJ, Simair RG, Smith K, Lewis N. Impact of prolonged sitting
17 on vascular function in young girls. *Experimental physiology*. 2015;100(11):1379-87.
- 18 25. Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, Padilla J. Prolonged sitting-
19 induced leg endothelial dysfunction is prevented by fidgeting. *American journal of*
20 *physiology*. 2016;311(1):H177-82.
- 21 26. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical
22 activity of work. *Lancet*. 1953;265(6796):1111-20.
- 23 27. Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. *Rev Endocr*
24 *Metab Disord*. 2013;14(1):5-12.
- 25 28. Owen N, Bauman A, Brown W. Too much sitting: a novel and important predictor of chronic
26 disease risk? *British journal of sports medicine*. 2009;43(2):81-3.
- 27 29. Padilla J, Sheldon RD, Sitar DM, Newcomer SC. Impact of acute exposure to increased
28 hydrostatic pressure and reduced shear rate on conduit artery endothelial function: a limb-
29 specific response. *American journal of physiology*. 2009;297(3):H1103-8.
- 30 30. Parsons TJ, Sartini C, Ellins EA et al. Objectively measured physical activity and sedentary
31 behaviour and ankle brachial index: Cross-sectional and longitudinal associations in older
32 men. *Atherosclerosis*. 2016;247:28-34.
- 33 31. Peddie MC, Bone JL, Rehner NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting
34 reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover
35 trial. *The American journal of clinical nutrition*. 2013;98(2):358-66.
- 36 32. Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on
37 lower and upper limb micro- and macrovascular dilator function. *Experimental physiology*.
38 2015;100(7):829-38.
- 39 33. Restaino RM, Walsh LK, Morishima T et al. Endothelial dysfunction following prolonged
40 sitting is mediated by a reduction in shear stress. *American journal of physiology*.
41 2016;310(5):H648-53.
- 42 34. Sena CM, Pereira AM, Seica R. Endothelial dysfunction - a major mediator of diabetic
43 vascular disease. *Biochim Biophys Acta*. 2013;1832(12):2216-31.
- 44 35. Shuval K, Finley CE, Barlow CE, Gabriel KP, Leonard D, Kohl HW. Sedentary behavior,
45 cardiorespiratory fitness, physical activity, and cardiometabolic risk in men: the cooper
46 center longitudinal study. In: *Proceedings of the Mayo Clinic Proceedings*. 2014. p. 1052-62.
- 47 36. Stamatakis E, Hamer M, Tilling K, Lawlor DA. Sedentary time in relation to cardio-metabolic
48 risk factors: differential associations for self-report vs accelerometry in working age adults.
49 *Int J Epidemiol*. 2012;41(5):1328-37.

- 1 37. Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of prolonged sitting and
2 breaks in sitting time on endothelial function. *Medicine and science in sports and exercise*.
3 2015;47(4):843-9.
- 4 38. Thosar SS, Bielko SL, Wiggins CC, Klaunig JE, Mather KJ, Wallace JP. Antioxidant vitamin C
5 prevents decline in endothelial function during sitting. *Med Sci Monit*. 2015;21:1015-21.
- 6 39. Thosar SS, Bielko SL, Wiggins CC, Wallace JP. Differences in brachial and femoral artery
7 responses to prolonged sitting. *Cardiovasc Ultrasound*. 2014;12:50.
- 8 40. Thosar SS, Johnson BD, Johnston JD, Wallace JP. Sitting and endothelial dysfunction: the role
9 of shear stress. *Med Sci Monit*. 2012;18(12):RA173-80.
- 10 41. Zhang N, Andresen BT, Zhang C. Inflammation and reactive oxygen species in cardiovascular
11 disease. *World J Cardiol*. 2010;2(12):408-10.

12

13

1 **FIGURE LEGENDS**

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

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

12

13 **TABLE LEGEND**

14 **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.