THE EFFECT OF SEDENTARY BEHAVIOUR ON CARDIOVASCULAR BIOMARKERS IN ACTIVE, HEALTHY ADULTS.

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A thesis submitted in partial fulfilment of the requirements of

Liverpool John Moores University for the degree of Master of Philosophy

September 2016

Abstract

Sedentary behaviour (SB) is an independent predictor of cardiovascular disease (CVD) morbidity and mortality, yet little is known about the effect of SB on markers of cardiovascular health in active adults. Therefore the aim of the study was to determine whether increased SB is associated with greater cardiovascular risk in an active population.

Twenty-six healthy participants (aged 27.9±8 years, 16 males) were recruited and visited the laboratory on two occasions. During visit one, body composition (waist and hip circumference and body fat percentage (BF%)) were measured, and following 15 minutes of supine rest, ultrasound assessment of brachial and femoral artery flow-mediated dilation (FMD) and carotid artery intima-media thickness was conducted. Carotid-femoral pulse wave velocity and middle cerebral artery blood flow velocity were assessed via applanation tonometry and transcranial Doppler ultrasonography respectively. Continuous blood pressure and carotid artery reactivity (via ultrasound) were recorded during the cold pressor test (CPT). During visit two, cardiorespiratory fitness (CRF; VO_{2peak}) was assessed using the modified Bruce protocol. Physical activity (PA) and SB was objectively measured for seven days via accelerometry and inclinometry respectively. Participants were grouped into tertiles according to sedentary time: LoSIT (n=9), MidSIT (n=9) and HiSIT (n=8) and univariate ANCOVAs determined the effect of SB on cardiovascular biomarkers across the groups. Data is presented as mean±standard deviation.

There was a significant difference between groups in hip circumference (LoSIT 96.9±3.2cm, MidSIT 99.3±4.8cm, HiSIT 106.3±7.9cm; *P*=0.026), BF% (LoSIT 17.81±5.80%, MiSIT 22.40±9.61%, HiSIT 27.60±7.11%; *P*=0.035) and VO_{2peak} (LoSIT 50.58±3.65ml/min/kg, MidSIT 47.52±9.1465ml/min/kg, HiSIT 39.75±7.5965ml/min/kg;

P=0.043). There was no effect of SB on vascular markers and CPT responsiveness (*P*>0.05).

In conclusion, in an active population, SB appears to be detrimentally associated with CRF and body composition, whilst exceeding the guidelines of 150 minutes of moderate-vigorous or 75 minutes of vigorous intensity PA per week appears to be protective of the vasculature.

Acknowledgements

Firstly I would like to express my sincere gratitude to my director of studies, Dr. Nicola Hopkins for putting up with me asking a million questions a day, and for her continued support and guidance, including training me in the lab as well as throughout my data analysis and write up. Also I would like to thank my co-supervisors Dr Lee Graves for providing accelerometry training and helping me recruit through the health checks database, which proved very successful. Thirdly, thanks to Dr David Low helping me get my head around autonomic function and for technical support on LabChart.

A massive thank you to the other members of Team FMD (Joe Maxwell and Sophie Carter) for being guinea pigs to practice ultrasound amongst everything else on them before I started data collection. A special thank you Sophie C for helping me in the lab during data collection, as some of the tests couldn't be done on my own. Furthermore, the remaining postgraduates in the office deserve a mention for providing banter and laughs during days of reading and typing. Who wants to work in a silent office, anyway? I am also very appreciative of the technicians Gemma and Dean for being there when the equipment played up – rarely such a thing as a smooth, problem-free testing session!

Finally, I'd like to thank my family back at home for their continued support, and for spoiling me with a takeaway or a roast dinner whenever I go home for the weekend (I only have a microwave oven and 2-ring hob in my flat, so limited on what I can cook). I wouldn't be completing my MPhil and writing this thesis if it wasn't for my grandparents and parents supporting me financially as I couldn't get a bank loan – I will pay them back eventually!

Declaration

I declare that the work within this thesis is entirely my own.

Publications completed during the MPhil tenure

Carter, S, Hartman, Y, **Holder, S**, Thijssen, DH, Hopkins ND. Sedentary behavior and cardiovascular disease risk: mediating mechanisms. *Exercise and Sports Science Reviews,* in preparation.

Poster communications

S.M.Holder, L.E.F Graves, S.E. Carter, R.Hitchen, D.A.Low, & N.D. Hopkins. The effect of workplace sitting on vascular health in healthy adults. Faculty of Science Postgraduate Research Seminar and Poster Day 2016.

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List of Abbreviations

ANCOVA	Analysis of covariance
AusDiab	Australian Diabetes, Obesity and Lifestyle study
BF%	Body fat percentage
BMI	Body mass index
BP	Blood pressure
BRS	Baroreflex sensitivity
CAR%	Carotid artery reactivity
CBF	Cerebral blood flow
cIMT	Carotid intima-media thickness
CHD	Coronary heart disease
CPT	Cold pressor test
CRF	Cardiorespiratory fitness
CRP	C-reactive protein
CVCi	Cerebrovascular conductance index
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxide synthase
FMD	Flow-mediated dilation
GSH-Px	Glutathione peroxidase
HR	Heart rate
IPAQ	International physical activity questionnaire
LPA	Light physical activity
LPL	Low-density lipoprotein
MAP	Mean arterial pressure
MCAv	Middle cerebral artery blood flow velocity
METs	Metabolic equivalents
MPA	Moderate physical activity
MVPA	Moderate-vigorous physical activity
NO	Nitric oxide
PA	Physical activity
PWV	Pulse wave velocity
rAIx	Radial augmentation index
ROS	Reactive oxygen species

RPE	Rate of perceived exertion
SB	Sedentary behaviour
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
SR_{AUC}	Shear rate area-under-the-curve
TCD	Transcranial Doppler
TNFα	Tumor necrosis factor alpha
TV	Television
VO_{2peak}	Peak oxygen consumption
VPA	Vigorous physical activity

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CHAPTER 1

LITERATURE REVIEW

1.1 Physical Activity and Cardiovascular Disease Risk

Physical activity (PA) is defined as “any bodily movement produced by skeletal muscles that results in energy expenditure” (Caspersen et al., 1985) and the health benefits of engaging in PA are now well established (Warburton et al., 2006). The importance of PA in CVD risk reduction was first highlighted by Morris et al. (1953), when it was discovered that London bus drivers experienced more than double the amount of CVD incidences than bus conductors. The authors speculated that the PA undertaken by the bus conductors as part of their daily duties prevented the development of CVD. This seminal research was the first to highlight the need to understand the relationship between PA and health outcomes. Since this landmark study, many researchers have attempted to quantify the contribution of PA to CVD morbidity and mortality, culminating in a recent estimate which suggests that physical inactivity, defined as non-compliance with PA guidelines (Hallal et al., 2003), causes 6% of the global CVD burden (Lee et al., 2012).

Compliance with the global PA guidelines of 150 minutes of moderate-vigorous intensity PA (MVPA) or 75 minutes of vigorous intensity PA (VPA) per week (World Health Organisation, 2010), is beneficially associated with numerous traditional risk factors, including blood pressure and cholesterol (Hu et al., 2007). These factors contribute to the Framingham Risk score, which is algorithm to estimate 10-year risk of developing CVD, comprised of risk factors including cholesterol level, smoker status, diabetes and blood pressure. Although this has been widely used and useful to some extent, Mora et al. (2007) found that these traditional risk factors only accounted for 59% of disease risk. This presented a “risk factor gap”, in that the effects of PA on traditional risk factors does not account for the full extent of CVD risk reduction (Green et al., 2008). The remaining 41% left unaccounted for may be explained with the

inclusion of emerging/novel risk factors (Joyner & Green, 2009), including CRF (DeFina et al., 2013), arterial stiffness (Andersson et al., 2015), artery endothelial function (Green et al., 2003; Siasos et al., 2013), atherosclerosis (Kozakova et al., 2010) and autonomic function (Joyner & Green, 2009). The above guidelines are the minimum amount of exercise needed to maintain health, and additional activity provides further health benefits (World Health Organisation, 2010). Additionally, those who are physically active have a 20-30% reduced risk of all-cause mortality (Lee & Skerrett, 2001; Moore et al., 2012). Interestingly there is a curvilinear dose-response relationship between PA level and disease risk (Department of Health, 2004), with the greatest risk reduction occurring when shifting from inactive to low activity (Figure 1).

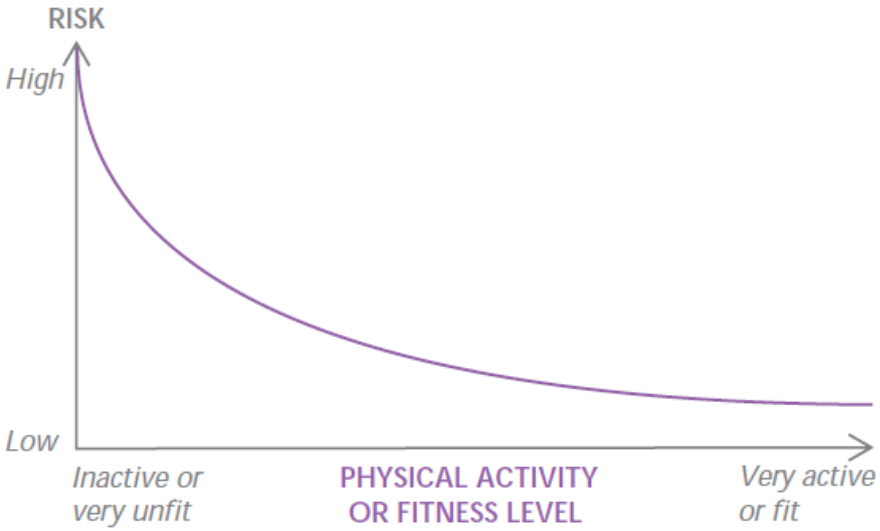

Figure 1: Dose-response relationship between PA and disease risk. (Department of Health, 2004).

1.2 Sedentary Behaviour

Sedentary behaviour (SB) is a different construct to physical inactivity, with both behaviours having different determinants (Pearson et al., 2014). Whilst SB is defined as “any waking behaviour with an energy expenditure of <1.5 METs while in a sitting

or reclining posture" (Tremblay, 2012), physical inactivity is, as stated previously, recognised by not meeting the PA guidelines (Hallal et al., 2003). Whilst the cardiovascular benefits of PA are well defined, the relationship between SB and CVD remains unclear. Over the last decade, SB has emerged as an independent risk factor for CVD, and includes occupational, transportation and leisure time behaviours. Prevalence of SB can be attributed to the reliance on the use of technology in many occupations (van Uffelen et al., 2010), as well as advances in entertainment technology e.g., computer game development, enhanced television (TV) viewing experience and increased usage of tablet devices (Hobbs et al., 2015).

1.2.1 Sedentary Behaviour and Cardiovascular Disease and Mortality Risk

The Australian Diabetes, Obesity and Lifestyle study (AusDiab), a population-based longitudinal study, examined the relationship between TV viewing time (a surrogate for SB) and diabetes, CVD risk factors, and mortality (Dunstan et al., 2010). The authors found that prolonged, uninterrupted periods (≥4 hours per day) of TV viewing was associated with increased risk of all-cause mortality, and for each additional hour of viewing time, all-cause mortality risk increased by 18%. This research provided the first epidemiological evidence that sitting time may increase disease risk, and has since been supported by further population-based studies (Wijndaele et al., 2011; Stamatakis et al., 2011). These initial studies highlighted the potential health risks associated with sitting, however due to limitations associated with correlation analysis, and the fact that TV viewing is commonly associated with increased calorie intake (Gore et al., 2003), direct independent inferences could not be made regarding SB and CVD risk.

Since these studies were conducted, researchers have attempted to better understand the relationship between SB and CVD by using subjective and objective methods to measure SB time. These data have consistently reported an association between prolonged periods of SB and CVD disease risk, independent of PA (Thorp et al., 2011; Chau et al., 2013b; Biswas et al., 2015) and have also begun to provide evidence that SB may have an independent effect on numerous markers of health including CVD risk and mortality.

The findings of the Women's Health Initiative Observational Study demonstrated that sitting for ≥10 hours per day was associated with significantly greater hazard ratio for CVD risk, compared to individuals engaging in <10 hours of sitting per day, independent of PA time (Chomistek et al., 2013). More recently, Seguin et al. (2014) reported a linear dose-response relationship between SB and all-cause mortality risk. Contrarily, a meta-analysis by Chau et al. (2013b) found a decrease in all-cause mortality risk when PA was taken into account, suggesting that PA somewhat mitigates the deleterious effects of SB. Whilst these findings indicate a relationship between SB and CVD, the mechanisms which underpin this relationship are poorly understood, and furthermore the question of independence of this relationship from PA remains unclear.

1.2.2 Sedentary Behaviour and Cardiometabolic Risk

As the deleterious health effects of SB on cardiometabolic and cardiovascular health have emerged (Tremblay et al., 2010), more attention has been given to this field of research. High levels of SB can coexist with high levels of MVPA, and associations between SB and cardiometabolic health outcomes are apparent even in individuals

who meet the PA guidelines (Healy et al., 2008b). Irrespective of exceeding the PA guidelines, the authors found a detrimental dose-response relationship between sitting time and cardiometabolic biomarkers such as body mass index (BMI), waist circumference, blood pressure (BP) and cholesterol. These findings imply that in addition to the promotion of 150 minutes of weekly MVPA, simultaneously reducing sedentary time may also be critical for the optimisation of cardiometabolic health. This has led to the "active couch potato hypothesis" where excessive SB is hypothesised to counteract at least some of the health benefits accrued by complying with the PA guidelines (Owen et al., 2010).

Bakrania et al. (2016) objectively measured PA and SB in a cohort of over 2000 participants, and categorised subjects based on PA, where active was defined as meeting PA guidelines: a) couch potatoes – inactive and high SB, b) light movers – inactive and low SB, c) sedentary exercisers - active and high SB and d) busy bees – active and low SB. Active individuals, including the sedentary exercisers had a more favourable cardiometabolic risk profile, including waist circumference and cholesterol, than couch potatoes. These results emphasise the importance of meeting the PA guidelines to protect against cardiovascular and metabolic disease.

1.2.3 Sedentary Behaviour and Cardiovascular Markers

Limited research has been conducted in order to determine the relationship between SB with surrogate markers of CVD risk as much of the focus has been on metabolic parameters (Carson et al., 2014; Crichton & Alkerwi, 2014; Bakrania et al., 2016). Therefore, little is known about the specific pathways involved in the development of sedentary-induced CVD, however it is likely that a combination of alterations in

pathways which regulate functional and structural vascular parameters, as well as cardiovascular autonomic control are implicated. Whilst the relationship between SB and metabolic variables has been investigated in active individuals (Healy et al., 2008b), the active couch potato hypothesis is yet to be examined using cardiovascular outcomes.

One potential mechanism through which SB may exert cardiovascular effects is a reduction in shear stress. Shear stress is the “tangential force derived by the friction of the flowing blood on the endothelial surface” (Giannoglou, 2008), and is a key regulator of endothelial function (Tinken et al., 2010). Initial findings demonstrate that as a result of low muscular activity associated with SB, blood flow (Thosar et al., 2012), and therefore shear stress are reduced, and consequently artery endothelial function is impaired (Malek et al., 1999).

A second mechanism which may link SB with the development of CVD, albeit indirectly, is poor CRF. A sedentary lifestyle is generally associated with poor CRF (Minder et al., 2014), which can be associated with autonomic nervous system imbalance, characterised by hyperactivity of the sympathetic, and decreased activity of the parasympathetic components of the autonomic nervous system (Thayer & Lane, 2007). This, in turn, puts greater tensile stress on the heart and vasculature, and increases risk of cardiovascular events (Giannoglou, 2008). Sympathetic hyperactivity causes an increase in resting heart rate (HR) and BP (Mourot et al., 2009), which in the long term, through the development of hypertension, increases CVD risk. Hypertension is also recognised to predict a decline in cerebral blood flow (CBF; Muller et al. (2012)). Although unknown, it could be hypothesised that these factors are sitting-induced and likely to be multifaceted and interlinked, therefore all contributing to the development of CVD.

1.3 Effect of Physical Activity and Sedentary Behaviour on Vascular Structure and Function

1.3.1 Carotid Intima-Media Thickness

Carotid artery intima-media thickness (cIMT), measured non-invasively using B-mode duplex ultrasound (Figure 2), is a surrogate marker of atherosclerosis (Paul et al., 2010), and predicts risk of cardiovascular events such as stroke and myocardial infarction (O'Leary et al., 1999; Thijssen et al., 2012). Atherosclerosis is an inflammatory disease of the arteries, whereby the artery wall thickens due to the build-up of substances such as cholesterol and smooth muscle cells (Cahill & Redmond, 2016). This ultimately results in a decrease in luminal diameter, restricting blood flow through the vessel. Artery wall thickening is thought to contribute to increases in arterial stiffness (Lakatta & Levy, 2003) and a decrease in brachial artery endothelial function (Juonala et al., 2004; Yao et al., 2014), suggesting that these biomarkers are all related to the atherosclerotic process (Koivistoinen et al., 2012).

The prognostic value of cIMT has been widely accepted for many decades as it provides a marker for increased risk of stroke, coronary heart disease (CHD) and death within 10–12 years (Bots et al., 1999). In the Atherosclerosis Risk in Communities Study (Chambless et al., 1997), the authors assessed the relationship between cIMT and CHD incidence over 4-7 years of follow-up from over 12000 healthy participants. A relationship was observed between cIMT and incident CHD, even after adjustment for other risk factors, including blood pressure, cholesterol and smoker status. Further longitudinal population-based research has predicted that even in a healthy population, an increase in cIMT of 0.1mm leads to a 10-15% increased risk of a future cardiovascular event (Lorenz et al., 2007; van den Oord et al., 2013). Together,

the above evidence proposes IMT as one of the underlying causes of cardiovascular event-related deaths.

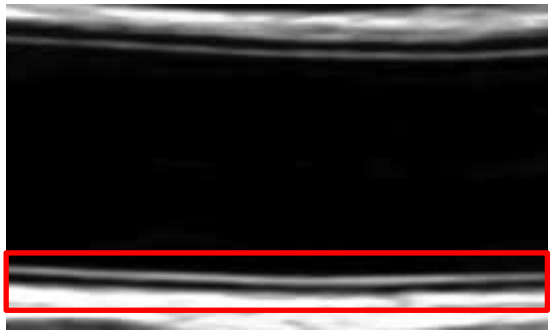

Figure 2: Ultrasound image of the carotid artery (cIMT visible on the bottom wall)

There is, however, inconsistent evidence regarding the association between PA and cIMT, though the majority of research demonstrates an inverse relationship between PA and cIMT. Umbreen et al. (2014) found that, according to PA scores from the International Physical Activity Questionnaire (IPAQ), individuals who fitted into the "moderate PA" category had a significantly smaller cIMT than those in the "low PA" category. Longitudinal research has also demonstrated an inverse relationship between PA and cIMT. Regular PA was inversely associated with the progression of cIMT during 6.5 year follow up (Palatini et al., 2011). Whilst the authors observed an increase in cIMT in both active and sedentary individuals, the rate of progression was significantly greater in sedentary subjects compared to their active counterparts. Similarly, Nordstrom et al. (2003) reported a strong inverse association between self-reported leisure time PA and cIMT. The authors observed a three-fold increase in progression of cIMT in sedentary compared to active individuals from baseline to 1.5- and 3-year follow up. Furthermore, research has investigated the role of PA intensity in the development of cIMT. Firstly, Kozakova et al. (2010) performed cIMT assessment in 495 participants at baseline and after 3 years, with a period of habitual

PA assessed via a combination of accelerometry and IPAQ. The authors found that after the 3-year follow up, subjects engaging in VPA had significantly lower progression of cIMT over time than those only engaging in light-moderate PA. Additionally, an inverse relationship was established between time engaged in vigorous or very vigorous PA and cIMT (Gomez-Marcos et al., 2014). Together, these results suggest that PA and PA intensity has a role to play in cIMT progression rate.

Recently, Diaz et al. (2016) assessed the relationship between self-reported leisure- (TV viewing time), non-leisure (occupational) sitting and cIMT. Interestingly, the authors found that longer TV viewing time (>2 hours per day) was associated with greater cIMT, whereas occupational sitting was associated with lower cIMT. These results could be attributed to the differences in accumulation of SB during TV viewing, i.e. more prolonged periods of sitting compared to perhaps frequent activity breaks during work hours. On the other end of the PA spectrum, cIMT has been positively associated with objectively measured total SB and sedentary time in ≥10 minute bouts (Garcia-Hermoso et al., 2015), suggesting that not only is total time spent in SB important, but the manner in which it is accumulated. Furthermore, Kozakova et al. (2010) objectively measured PA in a subgroup of healthy participant, and SB was found to be directly associated with cIMT at baseline, independent of age.

The underlying mechanisms of the relationship between PA, SB and cIMT, however, remain incompletely understood. PA is thought to positively affect cIMT through a positive impact on adiposity, serum lipoproteins and inflammation (Bertoni et al., 2009), which seems plausible as blood lipids and inflammatory markers contribute to the atherosclerotic process. Previous evidence suggests there is a strong correlation between inflammatory markers and IMT (Magyar et al., 2003). Evidence has demonstrated a positive association between SB and cIMT, when statistically

controlling for inflammatory markers (including C-reactive protein (CRP)), the association no longer existed (Garcia-Hermoso et al., 2015), which implies that markers of inflammation may be the mediating mechanism underpinning the relationship between SB and cIMT.

1.3.2 Endothelial Function

The vascular endothelium is the inner layer of the arterial wall exposed to direct contact with the blood stream, and is responsive to hormonal and hemodynamic stimuli. Its function is to regulate platelet function, vascular smooth muscle cell growth and vascular tone via the synthesis and release of vasoactive substances (Cahill & Redmond, 2016). The key vasoactive substance released is nitric oxide (NO), which is a potent vasodilator with anti-atherogenic properties to maintain vessel homeostasis (Bloodsworth et al., 2000). Endothelial function is essential for vascular health and vasomotor control (Green et al., 2004) and endothelial dysfunction has been established as an early surrogate marker of CVD (Vita & Keaney, 2002; Deanfield et al., 2007), which appears to contribute to the development and/or acceleration of the atherosclerotic process (Bonetti et al., 2003). A frequently-used, non-invasive technique to examine endothelial function in humans in vivo is flow-mediated dilation (FMD; Anderson (2007)). Brachial artery endothelial function, measured using the FMD test, is an established predictor of CVD. It is well documented that PA and greater CRF are associated with enhanced endothelial function (Hagg et al., 2005; Davison et al., 2010; Siasos et al., 2013), through greater bioavailability of NO (Gliemann et al., 2014). The proposed mechanism thought to mediate the beneficial effects of PA on endothelial function is an exercise-induced increase in blood flow, causing an increase in laminar shear stress, which initiates endothelial NO synthase (eNOS)

phosphorylation (Thosar et al., 2012), resulting in NO production and thus availability (Laughlin, 2004), facilitating vasodilation and anti-atherogenic properties of the artery.

Acute, prolonged SB is characterised by low muscular activity, decreased blood flow and, consequently, reduced cyclical shear stress on artery endothelial cells, resulting in a down-regulation of endothelial function (Thosar et al., 2012). This role of shear stress and blood flow during prolonged SB has recently been tested experimentally by Restaino et al. (2016), the authors compared popliteal artery FMD after 3 hours of sitting with one foot submerged in hot water to increase blood flow and shear stress, whilst the opposite foot stayed dry (control). The results showed impaired blood flow, shear and FMD in the control leg, whilst flow, shear and endothelial function were maintained in the heated leg, implying that the sitting-induced decline in popliteal artery endothelial function is caused by reductions shear stress. Similarly, Thosar et al. (2014) also found a significant decline in femoral artery FMD in response to 3 hours of uninterrupted sitting. However, breaking up sitting with a 5 minute light intensity walk every hour prevented this decline (Thosar et al., 2015a), as did a similar study design in children (McManus et al., 2015), where breaking up a 3-hour sitting period with 10 minutes of moderate intensity cycling every hour prevented the decline in femoral artery FMD observed in the sit condition. Similar results, but in the popliteal artery was observed when the sitting period was extended to 6 hours and function appeared to be fully restored after a 10 minute walk at a self-selected pace at the end of the sitting period (Restaino et al., 2015). Although blood flow and thus shear was reduced over the course of the sitting period, interestingly, the decline in FMD observed in the above studies was not accompanied by changes in shear rate area-under-the-curve during reactive hyperemia. This suggests that diminished shear stress stimulus was not responsible for the blunted FMD response. The authors have collectively hypothesised

potential mechanisms, including blood viscosity, which has previously been associated with low shear rates (Ku et al., 1997, as cited in Thosar et al. (2015a)) as observed in the above studies. Furthermore, viscosity has been shown to be greater in the lower limbs following 2 hours of uninterrupted sitting (Hitosugi et al., 2000), further suggesting a potential role in the attenuation of FMD. In summary, whilst it is clear that acute bouts of SB attenuate endothelial function, further research is required to establish the relationship between habitual SB and endothelial function in adults, and interactions with PA.

1.3.3 Arterial Stiffness

Arterial stiffness, or reduced compliance, describes the artery's inability to "expand and recoil" with the cardiac cycle (Tanaka et al., 2000) and is a recognised risk factor for CVD and all-cause mortality (Yambe et al., 2004). A meta-analysis of 17 longitudinal studies, including over 15000 participants concluded that an increase in PWV by $1m.s^{-1}$ was associated with increases of 47%, 47%, and 42% in cardiovascular events, cardiovascular mortality and all-cause mortality respectively (Vlachopoulos et al., 2010). Stiffening of the artery is most commonly associated with aging (Lakatta & Levy, 2003), and dementia (Blacher et al., 1998; Zieman et al., 2005; Vlachopoulos et al., 2010), and can be a result of increased IMT, endothelial dysfunction and changes in the structural and cellular properties of the arterial wall (Figure 3; Zieman et al. (2005)).

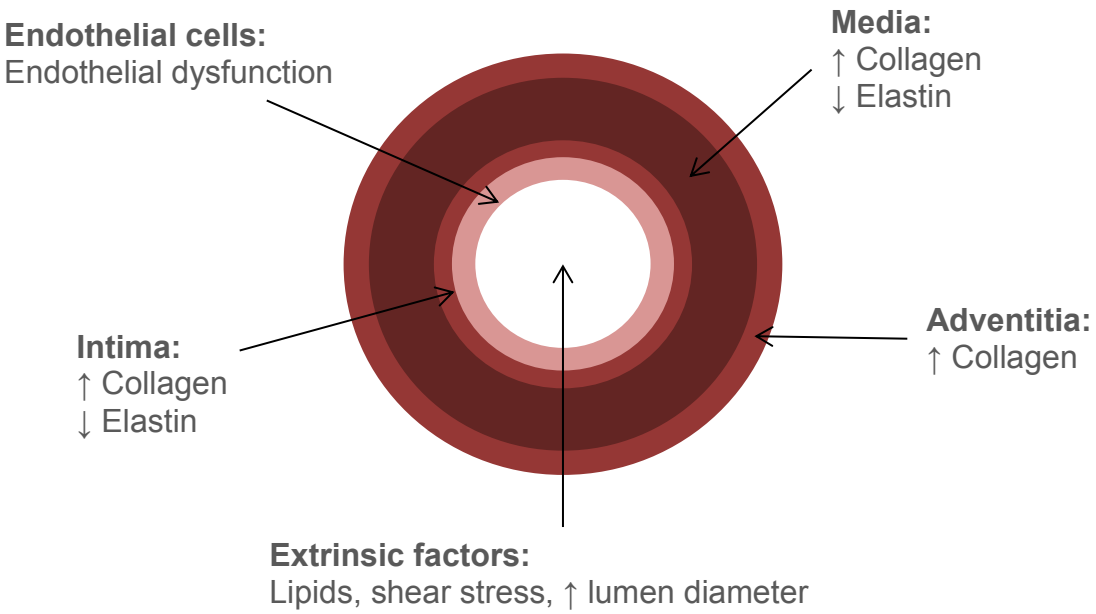

Figure 3: Summary of structural and cellular changes leading to arterial stiffness.

Collagen and elastin are the main proteins providing structural integrity and elasticity respectively, and the levels of each are maintained through a constant process of production and degradation. An imbalance of these proteins, caused by inflammatory markers (Zieman et al., 2005) or hypertension (Xu et al., 2000), leads to an overproduction of collagen and attenuation in elastin, which contributes to stiffening of the artery (Johnson et al., 2001).

Arterial stiffness is measured using applanation tonometry, which produces pulse wave velocity (PWV), and is considered the gold standard measure due to its feasibility and reproducibility (Van Bortel et al., 2012). The faster the pulse wave travels through the vessel (i.e. greater PWV), the stiffer the artery (Lakatta & Levy, 2003), where a "normal" value of PWV in healthy, normotensive individuals has been found to be approximately 6.1 ± 1.4 $m \cdot s^{-1}$ (Muller et al., 2013).

Increased stiffness can be slowed down and/or prevented through regular PA (Tanaka et al., 2000). Despite this established relationship, causality cannot be inferred, and the mechanisms are currently unknown. Firstly, Andersson et al. (2015) investigated the relationship between MVPA and PWV. All participants met ≥150 minutes MVPA per week guidelines and were categorised based on bout duration: a) MVPA accumulated in bouts of ≥10 minutes, and b) regardless of bout duration. The results showed a significant inverse relationship between MVPA and PWV, irrespective of bout duration. In contrast, the authors found no significant association between light intensity PA or SB and PWV. Furthermore, Garcia-Ortiz et al. (2014) evaluated arterial stiffness using radial augmentation index (rAIx), a surrogate measure of arterial stiffness (Nurnberger et al., 2002) and found that VPA was inversely associated with rAIx. Similarly, Gomez-Marcos et al. (2014) reported that total PA and time engaged in moderate, vigorous or very vigorous PA had an inverse relationship with stiffness, suggesting that, similar to cIMT, the intensity of PA is important for vascular health.

Boreham et al. (2004) discovered a significant inverse relationship between CRF and arterial stiffness in young adults. Interestingly, only sports-related PA (defined as activities of greater intensity than those undertaken during leisure time, such as swimming or tennis), but not leisure- and workplace PA, was found to be inversely associated with arterial stiffness. The authors suggested that the potential mechanism mediating the relationship between CRF and stiffness could be the low resting HR associated with greater fitness as a result of regular endurance-based PA and training. Although unknown, HR has been suggested to have an impact on structural properties of the arterial wall; a high resting HR may increase mechanical forces including BP and shear stress against the arterial wall, which in turn may lead to increased stiffness due to growth in vascular smooth muscle cells (Quan et al., 2014a). Recently, Lessiani

et al. (2016) examined the effect of an 8 week high-intensity exercise training programme on PWV in sedentary subjects (performing regular aerobic exercise 3 times/week and for 20 minutes/session, with a sedentary occupation) and found a significant increase in CRF alongside significant decreases in PWV and plasma triglycerides following training. These results suggest that the relationship between PA and PWV could be partially mediated through CRF, though there is, to my knowledge, no data available that shows a PA-induced improvement in PWV in the absence of improvements in fitness.

Whilst the majority of research is focussed on PA and stiffness, a few studies have reported a positive association between SB and stiffness (Duren et al., 2008; Horta et al., 2015), independently of PA and CRF (Quan et al., 2014b). Duren et al. (2008) compared PWV values across 3 groups: those who a) practiced yoga at least 2 days/week, b) performed ≥30 minutes aerobic exercise ≥3 days/week, or c) sedentary (defined by performing one or fewer bouts of vigorous aerobic activity a week in the previous year). The authors found significantly greater PWV values in the sedentary group compared to the aerobic and yoga groups, which suggests the presence of a relationship.

Interestingly, self-reported sitting time per weekend day, but not per weekday, was correlated with arterial stiffness in a sample of 2328 young (aged 26-36 years) adults (Quan et al., 2014b), perhaps due to weekend activity being a better reflection of discretionary sitting behaviour, whilst weekday sitting is mediated mostly by workplace demands. Furthermore, Horta et al. (2015) assessed PA across 24-hour cycles, and ordered participants into quartiles according to their sedentary time. The highest quartile of sedentary time (739.8-952.1 minutes/day, or 12.3-15.9 hours/day) had 0.39 m/s higher PWV than the lowest quartile (317.8-623.9 minutes/day, or 5.3-10.4

hours/day). However, sleep duration, which is a recognised CVD risk factor (Hoevenaar-Blom et al., 2014), has been taken into account. As “normal” sleep duration is considered to be between 7-8 hours (Hoevenaar-Blom et al., 2011), it would suggest that some participants in the lowest sedentary quartile (with lowest threshold of 5.3 hours) have poor sleep quality, which would put them at greater cardiovascular risk before stiffness is accounted for. Therefore, from these findings it is difficult to infer the true effects of SB on stiffness. Together the findings from the above studies imply that there is a relationship between SB and stiffness, however further research is required to strengthen this relationship by focussing on SB during waking hours and to offer mechanistic explanations.

1.4 Effect of Physical Activity and Sedentary Behaviour on Cardiovascular Autonomic Function and Cerebral Blood Flow

1.4.1 Autonomic Nervous System

The autonomic nervous system comprises of a sympathetic (adrenergic) and parasympathetic (vagal) component, responsible for stimulation/energy release and recovery respectively. The mechanisms involved across both components include the arterial baroreceptors, located in the aorta and carotid arteries, which detect changes in BP, and regulate HR accordingly (Wada et al., 2014). The sympathetic nervous system (SNS) is closely linked with the cardiovascular system, of which over-activity can lead to detrimental structural and functional changes to the vasculature (Marina et al., 2016), such as increased cIMT (Kadoya et al., 2015) and arterial stiffness (Bruno et al., 2012). Sympathetic over-activity causes an increase in BP, which in turn could

influence vascular structure and tone, via the stiffening of the carotid artery and aorta, and result in reduced baroreceptor responsiveness (or baroreflex sensitivity; BRS) to changes in BP (Monahan et al., 2001; Lenard et al., 2005). A reduction in BRS is associated with impaired regulation of BP, electrical instability of the heart, and therefore an increased risk of CVD morbidity and mortality (Skrapari et al., 2007).

1.4.1.1 Autonomic Function

The “risk factor gap” proposed by Joyner & Green (2009) included the importance of autonomic function on CVD risk

Autonomic function can be assessed using the cold pressor test (CPT), which is a useful predictor of future hypertension (Wood et al., 1984; Menkes et al., 1989). The test consists of measuring SBP, diastolic BP (DBP) and HR in response to an external cold stimulus, i.e. immersion of the hand/foot in a bucket of icy water for 3 minutes. In healthy individuals, the cold stress causes a sympathetic response, with an increase in BP over time, whilst HR responses vary between individuals (Mourot et al., 2009). The reason for this difference in HR responses is currently unclear as it could be attributed to the participant’s breathing pattern as well as muscle sympathetic nerve activity, both of which were not measured in the above study. The difference between baseline and the peak BP during immersion is then calculated as BP reactivity. Greater BP reactivity, or being a “hyper-reactor” has been linked to a greater risk of developing hypertension and CVD (Zhao et al., 2012). Currently however, there are no definitive guidelines regarding a “normal” reaction to the CPT, and different research groups use different thresholds to define a normal reactor compared to a “hyper-reactor” (Kasagi et al., 1995).

In addition to BP reactivity during the CPT, carotid artery reactivity (including diameter and blood flow velocity) can be measured via ultrasound as an index of coronary artery disease risk (Rubenfire et al., 2000). In healthy subjects, the carotid artery vasodilates during exposure to the cold stimulus, and is strongly correlated with cIMT (Rubenfire et al., 2000). Unpublished data from the laboratory at Liverpool John Moores University shows that CAR% reflects the response of coronary arteries, and that presence of traditional cardiovascular risk factors is associated with lower CAR%. Furthermore, CPT-induced coronary artery constriction is a predictor of future cardiovascular events (Schachinger et al., 2000) and given the relationship with carotid responses and feasibility of conducting the assessment, the CAR% test has potential as a valuable cardiovascular risk assessment. However, further research is required into the relationship between PA, SB and carotid artery reactivity.

1.4.1.2 Sedentary Behaviour, Physical Activity and Autonomic Function

A predominantly sedentary lifestyle is associated with autonomic imbalance, thus increased risk of morbidity and mortality (Thayer & Lane, 2007). Whilst research linking PA, SB and autonomic function is limited, available evidence suggests that some relationship exists.

In sedentary men, Monahan et al (2000) found that BRS progressively decreased with increasing age from 18–79 years but was 40-75% higher in active middle-aged and older men than their sedentary counterparts, suggesting that regular PA prevents the age-associated decrease in BRS. Zhang et al. (2013) investigated risk factors associated with BP response to the CPT and found that in addition to gender, age and weight status, self-reported physical inactivity was found to be a confounder

associated with greater BP reactivity. On the other hand, Bond et al. (2001) compared CPT (foot) responses in active vs inactive young adult African-American males. The authors found similar responses between groups, suggesting that PA status and resultant CRF may not contribute to the BP response to the CPT. However these results must be carefully considered as ethnicity may play a role in these differing outcomes (Calhoun et al., 1993).

1.4.2 Cerebral Blood Flow

The brain has the capability to maintain levels of blood flow in order to provide a constant supply of essential nutrients and oxygen, despite changes in BP (Querido & Sheel, 2007), constantly receiving around 15% of cardiac output (Lassen, 1959). Reductions in CBF can negatively impact cognitive function (Byrne & Byrne, 1993) and stroke risk (Gertz et al., 2006). Heart failure patients experience up to 31% decline in CBF compared to healthy individuals (Gruhn et al., 2001), due to the resultant diminished cardiac output. Traditional cardiovascular risk factors including Framingham risk score and appear to be negatively associated with mean and pulsatile CBF, leading to cerebral pathology and cognitive impairment (Pase et al., 2012). These results imply that cardiovascular function is closely related to CBF and cerebrovascular disease risk.

Ainslie et al. (2008) were the first authors to investigate the effect of training status (endurance trained compared to sedentary, defined by engaging in vigorous aerobic-endurance exercise more than 4 times/week and competing in road running/cycling races and no regular PA respectively) on CBF. The authors discovered that the trained group had greater CBF compared to their sedentary counterparts, and this was

consistent across a broad age range of 18-79 years. Furthermore, Brown et al. (2010) found that older women (~65 years old) who had greater CRF had a lower resting BP and greater CBF compared to sedentary women of the same age group. From this, it is clear that the benefits of regular PA on the systemic vasculature are apparent in the cerebral vasculature and result in improved CBF and therefore a decrease in cerebrovascular disease risk (Bailey et al., 2013). These data highlight the importance of being physically active to maintain aerobic fitness, in order to counteract the age-related decline in cerebrovascular health. Despite the evidence showing that regular PA positively impacts CBF and health, the relationship between SB and CBF is yet to be established (Zlatar et al., 2014).

1.5 Summary and Aim

Whilst cardiovascular health benefits of habitual PA are widely established, limited research indicate a relationship between SB and some cardiovascular parameters including artery structure (cIMT and arterial stiffness). However, further research is required to determine associations between habitual SB and endothelial function, autonomic function measured by CPT responsiveness and CBF. Previous research has been conducted to investigate the effect of SB on cardiometabolic parameters in an active adult population (Healy et al., 2008b), but to our knowledge, this has not been replicated in cardiovascular markers. The aim of the investigation is therefore to determine whether increased SB is associated with markers of greater cardiovascular risk in a highly active population.

CHAPTER 2

THE EFFECT OF SEDENTARY BEHAVIOUR ON CARDIOVASCULAR BIOMARKERS IN ACTIVE, HEALTHY ADULTS

2.1 Introduction

Sedentary behaviour (SB) is an emerging cardiovascular risk factor, yet the relationship between SB and CVD risk is yet to be fully understood. Previous research has demonstrated an association between SB and cardiometabolic and to a lesser extent cardiovascular parameters including blood glucose, insulin and body composition (Healy et al., 2008b) CRF (Minder et al., 2014), autonomic imbalance (Thayer & Lane, 2007) artery stiffness (Quan et al., 2014b), wall thickness (Kozakova et al., 2010) and endothelial dysfunction (Thosar et al., 2014), all of which are established risk factors for CVD. Whilst evidence of associations is emerging, it remains unclear how PA interacts with this relationship and whether the relationship between these markers and SB is independent or mediated by PA.

The health benefits of PA are established but recent evidence suggests that high amounts of SB can be counteractive. A combination of meeting the government PA guidelines (World Health Organisation, 2010) and engaging in high amounts of SB or being an “active couch potato” is hypothesised to counteract the cardiometabolic health benefits accrued by complying with the PA guidelines (Owen et al., 2010).This was first highlighted by Healy et al. (2008b) by investigating the effect of TV viewing, as a surrogate for SB, on cardiometabolic health in an active population. The authors found a positive association between TV viewing and metabolic variables such as waist circumference, fasting and 2-hour plasma glucose, triglycerides, and high-density-lipoprotein cholesterol. This supports the idea that SB exerts an independent effect on the measured variables, and that compliance with PA guidelines, when coupled with high SB, may be insufficient to maintain some components of health. However, research of this nature has not been conducted for cardiovascular biomarkers, therefore the aim of the investigation is to determine whether increased

SB is associated with greater cardiovascular risk in a highly active population. It was hypothesised that individuals engaging in greater amounts of SB would be at greater cardiovascular risk than those in engaging in less SB.

2.2 Methods

2.2.1 Participants

Twenty six healthy participants (16 males) were recruited for the study. All participants were non-smokers, had no history of CVD, were not taking any form of medication. Females experienced a regular menstrual cycle and were not on any form of hormone-based contraception or hormone replacement therapy. Each participant provided written informed consent before taking part in the experimental procedure. The research study was ethically approved by the Liverpool John Moores School of Sport and Exercise Science Research Ethics Committee and adhered to the Declaration of Helsinki.

2.2.2 Experimental Procedure

Participants were required to visit the temperature controlled (20-22 °C) laboratory on 2 separate occasions. During the first visit subjects completed a battery of cardiovascular risk assessments, and in preparation, participants abstained from exercising for 24 hours and alcohol for 12 hours, as well as any food/caffeine/stimulants 6 hours prior to the experiment. All visits were completed at the same time of day (between 8-11am). A treadmill based CRF test (VO_{2peak}) was completed on the second visit (within 7 days of the first visit). Participants were advised

to eat a small amount and be well rested in preparation. Physical activity and SB were then objectively monitored for 7 consecutive days. Females completed the first visit within the first seven days of their menstrual cycle when oestrogen is lowest, in order to control for hormonal influences on the cardiovascular system (Liu et al., 2016).

2.2.3 Visit 1: Cardiovascular Assessments

2.2.3.1 Anthropometry and Body Composition

Stature and weight were recorded to the nearest 0.1unit using a stadiometer and digital scales respectively. Body mass index (BMI) was calculated as weight in kilograms divided by stature in metres squared (kg/m^2). Waist and hip circumference was measured three times to the nearest 0.1cm by the same researcher at the narrowest point around the waist and widest point around the hips, using athropometric measuring tape and an average of each was calculated. Waist:hip ratio was calculated as waist circumference divided by hip circumference. Body fat percentage (BF%) was estimated using bioelectrical impedance analysis (Tanita BC-420MA, Tanita Corp., Tokyo, Japan). Participants stood barefoot over the metal plates, and an electrical current was passed through the body. This current easily passes through water, but is met with resistance in fatty tissue, also known as impedance. The scale then uses inbuilt, validated equations (Jebb et al., 2000) to calculate BF%.

2.2.3.2 Vascular Assessments

After 20 minutes of supine rest, the following vascular markers were measured by the same trained researchers:

2.2.3.2.1 Carotid Intima-Media Thickness

Left common carotid artery diameter and far-wall intima-media thickness was assessed using high-resolution B-mode ultrasound (Terason u-smart 3300, Teratech; Figure 2) from three angles (approximately 45º, 90º and 135º). Once an optimum image was obtained, with clear contrast between the artery walls and lumen and distinction of IMT on the far wall, a period of 30 - 40 seconds was recorded from each angle. All measurements were performed by the same sonographer, and with the participant's head flat on the bed (no pillow) and neck slightly extended to allow for optimal imaging.

From the 30 – 40 seconds of cIMT recording, the best 6 - 8 seconds of clear IMT and artery walls was selected for analysis using IMT v3.0 edge detection software. An optimal region of interest, including both vessel walls with a minimum length of 1cm, was selected by the sonographer and the software produced a frame-by-frame edge detection output of the near and far wall and far-wall media-adventitia interface. The distance from the line of the far wall (lumen-intima interface) and line of the media-adventitia interface was defined as IMT. Each frame was checked by the same sonographer, to ensure the diameter and IMT had been registered correctly, and any tracking mistakes made by the software were deleted. Continuous calculations by the software gave an average of the IMT and diameter recorded over the selected 6 – 8 second period. This was repeated for all three angles, and an average of the three angles was calculated and used in data analysis. Importantly, this method is reliable

and operator-independent, demonstrating high levels of precision and accuracy for estimating conduit artery diameter and wall thickness (Thijssen et al., 2011b).

2.2.3.2.2 Arterial Stiffness

Central (carotid-femoral) PWV was assessed using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia) in the supine position. Firstly, three brachial artery BP measurements were taken in succession (Dinamap V100, GE Medical Systems, Germany), and an average was calculated and entered into the software. A single applanation tonometer probe was used to obtain a proximal (carotid artery) and distal (femoral artery) pulse, recorded over 10 cardiac cycles. The QRS complex was measured simultaneously using electrocardiography (ECG). The time between the R wave of the ECG trace and the foot of the proximal waveform is subtracted from the time between the R wave and the foot of the distal waveform to obtain the pulse transit time. To determine the distance used for PWV, the distance from the proximal measurement site to the suprasternal notch was subtracted from the distance between the distal measurement site and the suprasternal notch using an anthropometric measuring tape. PWV was automatically calculated by the software by dividing the distance between the two arterial recording sites by transit time to provide an index of arterial stiffness. PWV measurements were made in triplicate and an average was calculated and used in data analysis.

2.2.3.2.3 Brachial- and Femoral FMD

Left brachial and right femoral artery diameter were assessed simultaneously via high-resolution 2D duplex ultrasound (Terason u-smart 3300 or Terason t3000, Teratech;

Figure 4) with a 10-12 MHz linear array probe. B-mode images were obtained and optimised, and the probe was held in the same position for the duration of the test. After 1 minute of baseline measurement, occlusion cuffs, connected to a rapid inflator (Hokanson, Bellevue, WA), placed around the right thigh, proximal to the patella, and the left forearm, distal to the humeral epicondyle, were inflated to a pressure of 220mmHg for 5 minutes. FMD was recorded for a further 3 minutes post cuff deflation (Thijssen et al., 2011a; Figure 5). The reliability and validity of this test largely depends on compliance with current guidelines (Thijssen et al., 2011a).

FMD data was analysed by a specialised custom-designed edge-detection and wall-tracking software (Dicom Encoder), of which the reproducibility and validity have been demonstrated elsewhere (Woodman et al., 2001). This software tracks the vessel walls and blood velocity trace in B-mode frames via pixel density and frequency distribution algorithm. An optimal region of interest to be analysed was selected by the sonographer, chosen on the basis of the quality of the image, in regards to clear distinction between the artery walls and lumen. The peak artery FMD was defined as the peak percentage change in artery diameter from baseline to during the 3 minutes post cuff release. The software automatically calculated the relative diameter change, time to peak (following cuff release) and shear rate area-under-the-curve (SR_{AUC}). Despite the initial region of interest selection being operator-determined, the remaining analysis was independent of operator bias.

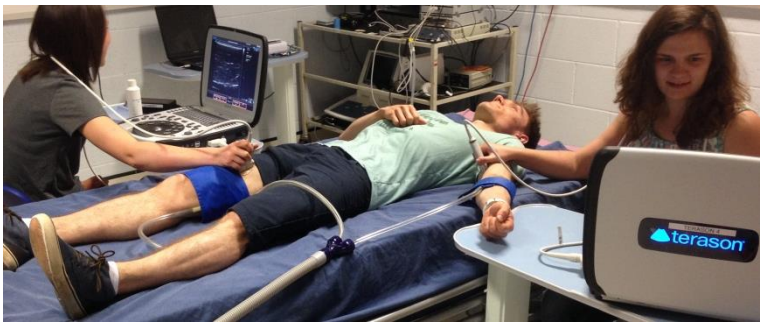

Figure 4: Simultaneous assessment of brachial- and femoral FMD

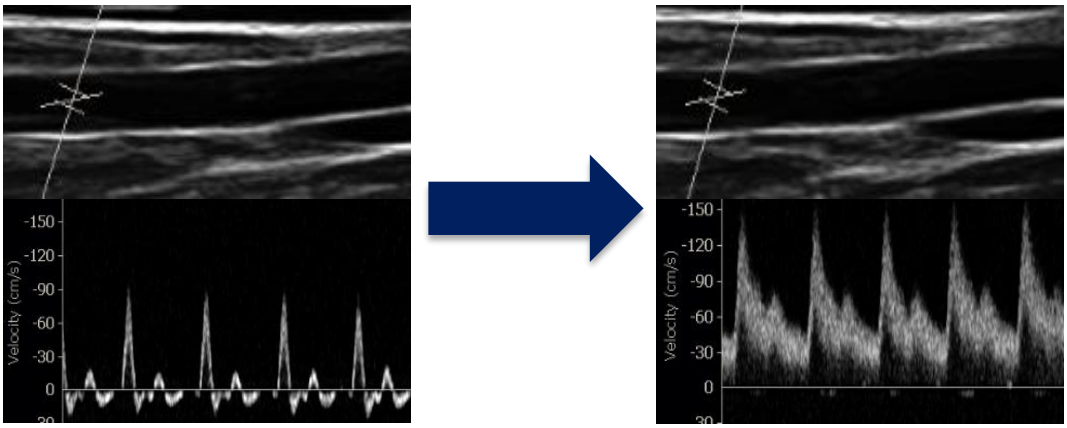

Figure 5: Ultrasound image of the brachial artery and blood flow velocity trace during baseline (left) and post cuff deflation (right).

2.2.3.2.4 Cerebral Blood Flow

Cerebral blood flow was measured at rest using transcranial Doppler (TCD) ultrasonography (DWL, Compumedics, Germany) from the middle cerebral artery (MCA). However, TCD measures blood flow velocity, rather than blood flow, as the vessel diameter is unknown, but providing the angle of the probes remain constant, blood flow velocity proves to be a valid and reliable index of blood flow (Giller et al., 1998; Ainslie & Duffin, 2009). TCD involves the subject wearing a headband, which supports an ultrasound probe on each side (Figure 6). Ultrasound gel was applied to the temporal window (area just above the zygomatic arch) and to the probes, and an optimal signal was obtained. To insonate the correct vessel, specific criteria was

followed regarding the mean and peak MCAv values as above 50cm.sec-1 and 80cm.sec-1 respectively, with the depth set between 40-60mm (Willie et al., 2011).

The raw MCAv data was extracted using LabChart Pro version 7 (ADInstruments, Australia), and the weighted mean MCAv was calculated from the peak envelope of the velocity trace (1/3 systolic + 2/3 diastolic), which accounts for the relative time spent in each phase of the cardiac cycle (Skow et al., 2013). Data was expressed as cerebrovascular conductance index (CVCi), which was calculated as MCAv divided by mean arterial pressure (1/3SBP + 2/3DBP; in this calculation, BP was taken from the average of the measurement preceding arterial stiffness).

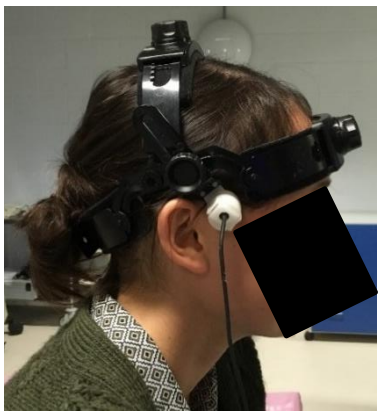

Figure 6: TCD headband

2.2.3.3 Autonomic Function

Continuous BP was measured by finger photoplethysmography (Finometer Pro, Finapres Medical Systems, Biomedical Instruments, Amsterdam: Netherlands). The cuff was placed around the mid-phalanx of the index/middle finger (dependent upon how well the cuff fitted) of the right hand. Simultaneously, R-R interval was assessed via 5-lead ECG and was displayed and recorded using PowerLab software (ADInstruments, Australia) for the duration of the following assessment:

2.2.3.3.1 Cold Pressor Test

Subjects were positioned supine on the bed, so that their left arm could easily drop off the side without further bodily movements, and with their neck slightly extended, allowing ultrasound assessment of carotid artery diameter and blood flow velocity response (carotid artery reactivity; CAR%) for the duration of the test. Once an optimal image was obtained, the test commenced with 1 minute of baseline. After the first minute, participants were instructed to drop their arm down and immerse their hand up to the wrist into a bucket of icy water (1-5ºC) for 3 minutes. For the duration of this test, participants were encouraged to breathe normally (avoid breath holding/hyperventilation) and keep as still as possible, without speaking. The CPT has been widely used for many years to assess cardiovascular reactivity to stress, which has been implicated as a reliable predictor of developing hypertension and CVD (Zhao et al., 2012). However, CAR% is not a widely established research method, therefore, to our knowledge, no reliability data is available.

BP (SBP and DBP) data was exported from LabChart Pro (ADInstruments, Australia) in 20 second intervals. BP reactivity was calculated as the difference between baseline BP compared to the peak BP recorded during hand immersion. CAR% was analysed using custom-designed edge detection software as previously described. Baseline diameter and blood flow were taken as an average from the 1 minute before hand immersion.

Post immersion data was assessed at 10-second intervals, and from this, peak diameter change (maximum dilation/constriction) and area under the curve for the diameter change during CPT (CAR_{AUC}) was calculated. The peak diameter change refers to dilation or constriction, and the direction of this change was determined by a positive or negative CAR_{AUC} (i.e. dilation or constriction respectively).

2.2.4 Visit 2: Cardiorespiratory Fitness Test

An incremental, treadmill-based test which involved walking and running until volitional exhaustion was used to determine maximum oxygen uptake (VO_{2peak}) in participants. VO_2 and VCO_2 were measured continuously, breath-breath via an online gas analysis system (Jaeger Oxycon Pro, Viasys Health Care, Warwick, UK). Heart rate (HR) was monitored continuously (Polar, Kempele, Finland). The Modified Bruce protocol was used, which started with a 2-minute slow walk, followed by 1-minute stages where the speed and incline gradually increased. During the final 20 seconds of each stage, participants were asked to rate their exertion, using the rate of perceived exertion (RPE) scale (Borg, 1970), and HR was recorded by the researcher. Criteria for participants reaching their maximal capacity was a respiratory exchange ratio >1.05 and/or heart rate >199 beats.min^{-1} and RPE at 20.

2.2.5 Physical Activity and Sedentary Behaviour Monitoring

Participants were required to wear an accelerometer and inclinometer on the right hip and right thigh respectively for seven consecutive days to assess habitual PA and SB.

2.2.5.1 Accelerometer

PA was objectively measured for 7 consecutive days using a hip-mounted tri-axial accelerometer (GT3X BT+ model, ActiGraph, Pensacola, Florida), of which the reliability has been demonstrated elsewhere (Aadland & Ylvisaker, 2015). Participants were instructed to wear the accelerometer during all waking hours, except during water-based activities, and to record the times at which they applied and removed the

device on a sheet provided. Work hours were also recorded, to allow work and leisure time activity to be distinguished during analysis

ActiLife software version 6.2 (ActiGraph, Pensacola, Florida) was used to initialise the device and download at 60-second epochs and analyse the raw data. Raw accelerometry data was presented in counts per minute (counts.min^{-1}). Non-wear time was defined as 90 consecutive minutes of zero counts.min^{-1} (Choi et al., 2011). PA intensity was determined using the following cut points (Sasaki et al., 2011): light (≤2689 counts.min^{-1}), moderate (≤6166 counts.min^{-1}), and vigorous (>6167 counts.min^{-1}). Inclusion criteria for analysis were ≥10 hours of wear time per day, for a minimum of 4 days, including one weekend day (Trost et al., 2005). Activity data was exported and handled in Excel (Microsoft) and total time (in minutes) spent in light, moderate and vigorous from the valid days (10 hours of wear time per day) was calculated. All participants met the inclusion criteria for PA data. To account for any differences in accelerometer wear time, PA data were also expressed as a percentage of wear time.

2.2.5.2 Inclinometer

Time spent in SB was monitored using an activPAL micro (PAL Technologies Ltd, Glasgow, UK). The activPAL is a lightweight, small (53 x 35 x 4 mm) tri-axial accelerometer, proven to provide valid and reliable data (Grant et al., 2006). The raw data determined sedentary (including sitting and lying), standing and stepping time, as well as step count and transitions from sedentary to standing. The monitor was worn on the middle-anterior line of the right thigh, concealed inside a rubber sleeve, and attached to the skin with a waterproof transparent film (Tegaderm Roll, 3M). This

allowed the device to be worn continuously for seven days, without the need for it to be removed for water-based activities or sleeping.

The device was initialised and raw data downloaded by 15 second epochs using activPAL software 7.2.32, then exported and handled using Excel (Microsoft, UK). The times recorded on the accelerometer and work hours log sheets as previously described were used in the analysis of the activPAL data to determine waking- and work hours respectively. In Excel, total seconds of sedentary time during waking- and work hours was summed and converted into minutes. Leisure sedentary time was calculated on the work days by subtracting work from waking hours sedentary time. Using the "Events XYZ" output file, the total number and average duration of sedentary bouts overall, and during work and leisure time were calculated. A sedentary bout was defined as no activity registered for at least 60 seconds, similar to (Healy et al., 2008a).

2.2.6 Statistical Analysis

Statistical analysis was conducted using IBM SPSS version 22 (SPSS Inc., Chicago, IL). Sex-specific differences across all variables were assessed via an independent t-test. Participants were then grouped according to average daily sedentary time; data was ranked by sedentary time, and split into tertiles (LoSIT n=9, MidSIT n=9, and HiSIT n=8). Differences between tertiles were assessed via analysis of covariance (ANCOVA) for all variables, controlling for sex and age. Statistical significance was recognised when a P value of <0.05 was observed. Data is presented as mean ± standard deviation.

2.3 Results

Twenty six participants (27.9±8 years old, BMI 23.9±2.8 kg/m2) were recruited to the study, of those, 19 had a full cardiovascular data set. One participant was unable to complete the CPT test, and a further four carotid artery scans were of poor quality, so were discarded. Furthermore, due to technical difficulties, brachial and femoral FMD data were missing for two participants. Breakdown of participant numbers for each measure with missing data is provided in Table 1.

2.3.1 Participant Characteristics

Males had significantly greater waist:hip ratio [+0.08, $P<0.001$], VO_{2peak} [+9.52ml/kg/min, $P=0.001$] and lower BF% [-14.12%, $P<0.001$]. Additionally males had a lower MCAv [-9.49cm/s, $P=0.04$] and CVCi [-0.16cm/s/mmHg, $P=0.017$] (Table 2). There were no significant differences between sexes for any PA data (from ActiGraph): LPA ($P=0.905$), MPA ($P=0.419$), VPA ($P=0.0.93$), and MVPA ($P=0.138$; Table 3). Similarly, there were also no differences for SB data (from activPAL): total sedentary time ($P=0.268$), total sedentary bouts ($P=0.288$), total bout duration ($P=0.454$), work sedentary time ($P=0.054$) and leisure sedentary time ($P=0.178$).

Table 1: Breakdown of participant numbers for each measure with missing data.

Variable	All	Males	Females	LoSIT	MidSIT	HiSIT
Brachial FMD	24	16	8	9	8	7
Femoral FMD	24	16	8	9	8	7
Cold Pressor ΔBP	23	16	8	8	9	6
Cold Pressor CAR	21	16	5	9	5	7

FMD – flow-mediated dilation; BP – blood pressure; CAR – carotid artery reactivity.

Table 2: Participant characteristics for all participants, and males and females independently.

	n	All	Males	Females
Age (y)	26	27.9 ± 8.0	28.4 ± 9.5	27.1 ± 4.9
Stature (m)	26	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1*
Weight (kg)	26	72.5 ± 10.3	74.2 ± 11.6	69.7 ± 7.7
BMI (kg/m^2)	26	23.9 ± 2.8	24.1 ± 3.2	23.9 ± 1.9
Waist circumference (cm)	26	79.4 ± 7.6	81.1 ± 8.6	76.7 ± 4.9
Hip circumference (cm)	26	100.6 ± 6.6	98.8 ± 6.8	103.6 ± 5.4
Waist:Hip ratio	26	0.79 ± 0.05	0.82 ± 0.04	0.74 ± 0.04*
Body fat (%)	26	22.41 ± 8.41	16.98 ± 4.79	31.10 ± 4.68*
VO_{2peak} (ml/min/kg)	26	46.2 ± 8.2	49.8 ± 7.5	40.3 ± 5.6*
Resting SBP (mmHg)	26	113 ± 10	115 ± 10	109 ± 9
Resting DBP (mmHg)	26	63 ± 8	64 ± 9	62 ± 5
MCAv (cm/s)	26	66 ± 11	63 ± 9	72 ± 11*
MAP (mmHg)	26	80 ± 8	81 ± 9	78 ± 6
CVCi (cm/s/mmHg)	26	0.84 ± 0.15	0.78 ± 0.11	0.94 ± 0.16*
PWV (m/s)	26	5.70 ± 0.72	5.82 ± 0.82	5.52 ± 0.51
Femoral FMD (%)	24	6.97 ± 3.32	7.05 ± 3.28	6.80 ± 3.62
Femoral Baseline Diameter (mm)	24	0.60 ± 0.07	0.62 ± 0.07	0.57 ± 0.05
Femoral SR_{AUC} (s, 10^4)	24	14.9 ± 7.6	14.9 ± 8.5	15.1 ± 6.3
Brachial FMD (%)	24	6.50 ± 2.66	6.45 ± 2.82	6.60 ± 2.50
Brachial Baseline Diameter (mm)	24	0.35 ± 0.05	0.38 ± 0.04	0.29 ± 0.03
Brachial SR_{AUC} (s, 10^4)	24	12.8 ± 8.1	11.1 ± 7.0	16.1 ± 9.6
cIMT (mm)	26	0.57± 0.06	0.56 ± 0.07	0.58 ± 0.04
Carotid Artery Diameter (mm)	26	6.2 ± 0.4	6.3 ± 0.3	6.2 ± 0.4
Cold Pressor ΔSBP (mmHg)	23	27 ± 12	28 ± 13	24 ± 12
Cold Pressor ΔDBP (mmHg)	23	18 ± 9	18 ± 9	16 ± 8
Cold Pressor CAR (%)	21	3.03 ± 5.31	3.45 ± 5.33	1.67 ± 5.63

MCAv – middle cerebral artery blood flow velocity; MAP – mean arterial pressure; CVCi – cerebrovascular conductance index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FMD – flow-mediated dilation; SR_{AUC} – shear rate area-under-the-curve; cIMT – carotid intima-media thickness; CAR – carotid artery reactivity.

* Indicates a significant difference between males and females ($P < 0.05$).

Table 3: Average daily physical activity and sedentary data for all participants, and males and females independently.

	All N = 26	Males N = 16	Females N = 10
ActiGraph data			
LPA (mins/day)	283 ± 72	281 ± 69	285 ± 81
MPA (mins/day)	56 ± 20	53 ± 20	60 ± 21
VPA (mins/day)	11 ± 11	8 ± 11	15 ± 9
MVPA (mins/day)	67 ± 22	62 ± 21	75 ± 22
LPA (%)	34.3 ± 8.8	34.1 ± 8.3	34.9 ± 10
MPA (%)	6.8 ± 2.4	6.5 ± 2.4	7.37 ± 2.5
VPA (%)	1.3 ± 1.3	1 ± 1.4	1.87 ± 1
MVPA (%)	8.1 ± 2.7	7.5 ± 2.5	9.24 ± 2.7
Accelerometer Wear Time (mins)	822 ± 102	827 ± 108	815 ± 98
ActivPAL data			
Sedentary Time (mins/day)	488 ± 73	477 ± 84	507 ± 52
Sedentary Bouts	31 ± 8	30 ± 9	33 ± 8
Total Bout Duration (mins)	18 ± 8	19 ± 9	16 ± 6
Work Sedentary Time (mins/day)	272 ± 111	238 ± 110	323 ± 95
Leisure Sedentary Time (mins/day)	222 ± 75	239 ± 73	197 ± 75

LPA – light physical activity; MPA – moderate physical activity; VPA – vigorous physical activity; MVPA – moderate-vigorous physical activity. PA and SB data was taken from the ActiGraph and activPAL monitors respectively. Sedentary bout = >1minute.

2.3.2 Tertile Analysis

Daily sedentary time was significantly different between each tertile (LoSIT 410.36±61.09mins, MidSIT 503.56±12.55mins and HiSIT 560.87±30.95mins; $P<0.001$, Table 4). There were no differences between groups in daily number of sedentary bouts ($P=0.882$), but total sedentary bout duration was significantly higher in HiSIT (22.75±13.95mins) than LoSIT (14.53±3.54mins; $P=0.049$), but not significantly different to MidSIT (17.69±2.82mins; $P=0.418$).

Workplace sedentary time was significantly higher in HiSIT (340.75±78.79mins) than LoSIT (186.10±84.36mins; *P*=0.003), but not MidSIT (304.99±108.05mins; *P*=0.450). Furthermore, MidSIT had significantly greater workplace sedentary time than the LoSIT (*P*=0.012). However, there were no differences between groups in leisure sedentary time (*P*=0.489). Additionally, no differences were observed for LPA (*P*=0.275), MPA (*P*=0.348), VPA (*P*=0.609), MVPA (P=0.687) and accelerometer wear time (*P*=0.720) between groups.

2.3.2.1 Body Composition and Cardiorespiratory Fitness

There was a significant effect of group on hip circumference (*P*=0.009), where HiSIT (106.3±7.9cm) had a significantly greater hip circumference than both MidSIT (99.3±4.8cm; *P*=0.022) and LoSIT (96.9±3.2cm; *P*=0.014, Table 5). HiSIT (27.60±7.11%) had significantly greater BF% than both MidSIT (22.40±9.61%; *P*=0.046) and LoSIT (17.81%±5.80%; *P*=0.014), however, there was no difference in BMI (*P*=0.112), waist circumference (*P*=0.164) or waist:hip ratio (*P*=0.994) across the groups. Those in HiSIT had a significantly lower VO_{2peak} (39.75±7.59ml/kg/min) than MidSIT (47.52±9.14ml/kg/min; *P*=0.025) and LoSIT (50.58±3.65ml/kg/min; *P*=0.03).

2.3.2.2 Vascular Assessments

There were no differences between groups for SBP (*P*=0.066), DBP (*P*=0.309), MAP (*P*=0.276), PWV (*P*=0.284), cIMT (*P*=0.158), brachial FMD (*P*=0.877) femoral FMD (*P*=0.947), MCAv (*P*=0.369) or CVCi (*P*=0.867, Table 5).

Table 4: Average daily physical activity and sedentary data across groups: LoSIT, MidSIT and HiSIT.

	LoSIT n=9 (2 females)	MidSIT n=9 (4 females)	HiSIT n=8 (4 females)
ActiGraph data			
LPA (mins/day)	313 ± 75	275 ± 71	257 ± 68
MPA (mins/day)	53 ± 17	51 ± 22	64 ± 21
VPA (mins/day)	11 ± 9	14 ± 13	8 ± 11
MVPA (mins/day)	64 ± 21	64 ± 22	73 ± 25
LPA (%)	39.4 ± 9.4	33.5 ± 8.6	30 ± 7.9
MPA (%)	6.7 ± 2.1	6.18 ± 2.7	7.5 ± 2.4
VPA (%)	1.3 ± 1.1	1.6 ± 1.5	0.9 ± 1.2
MVPA (%)	8.1 ± 2.7	7.8 ± 2.6	8.5 ± 2.8
Accelerometer Wear Time (mins)	793 ± 123	822 ± 99	856 ± 81
ActivPAL data			
Daily Sedentary Time (mins/day)	410 ± 61	503 ± 12*	560 ± 30*†
Daily Sedentary Bouts	31 ± 9	30 ± 6	32 ± 11
Total Bout Duration (mins)	14 ± 3	17 ± 2	22 ± 13*
Work Sedentary Time (mins/day)	186 ± 84†	304 ± 108*	340 ± 78*
Leisure Sedentary Time (mins/day)	207 ± 74	215 ± 79	251 ± 75

LPA – light physical activity; MPA – moderate physical activity; VPA – vigorous physical activity; MVPA – moderate-vigorous physical activity. PA and SB data was taken from the ActiGraph and activPAL monitors respectively. Sedentary bout = >1minute.

* Indicates significant difference compared to LoSIT ($P < 0.05$).

† Indicates a significant difference compared to MidSIT ($P < 0.05$).

2.3.2.3 Autonomic Function

There was no effect of group on SBP response (P=0.188), DBP response (P=0.858) or CAR% (P=0.548) during the CPT (Table 5).

Table 5: Participant characteristics across groups: LoSIT, MidSIT and HiSIT.

	n	LoSIT (n=9, 2 f)	MidSIT (n=9, 4 f)	HiSIT (n=8, 4 f)
Age (years)	26	25.2 ± 5.8	28.4 ± 6.8	30.3 ± 10.9
Stature (m)	26	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	26	68.5 ± 5.7	71 ± 4.7	78.5 ± 15.9
BMI (kg/m^2)	26	23.1 ± 1.7	23.2 ± 1.9	25.9 ± 3.7
Waist circumference (cm)	26	77.2 ± 6.3	77.9 ± 4.7	83.6 ± 10.4
Hip circumference (cm)	26	96.9 ± 3.2	99.3 ± 4.8	106.3 ± 7.9*†
Waist:Hip ratio	26	0.80 ± 0.06	0.79 ± 0.04	0.79 ± 0.07
Body fat (%)	26	17.81 ± 5.80	22.40 ± 9.61	27.60 ± 7.11*†
VO_{2peak} (ml/min/kg)	26	50.6 ± 3.6	47.5 ± 9.1	39.7 ± 7.6*†
MCAv (cm/s)	26	65.44 ± 8.26	64.44 ± 11.82	69.88 ± 12.83
MAP (mmHg)	26	81 ± 8	76 ± 5	83 ± 9
CVCi (cm/s/mmHg)	26	0.81 ± 0.11	0.85 ± 0.17	0.86 ± 0.19
Resting SBP (mmHg)	26	115 ± 9	109 ± 7	113 ± 14
Resting DBP (mmHg)	26	64 ± 9	60 ± 5	67 ± 7
PWV (m/s)	26	5.71 ± 0.79	5.49 ± 0.64	5.93 ± 0.75
Femoral FMD%	24	6.74 ± 3.51	7.78 ± 3.69	6.33 ± 2.90
Femoral Baseline Diameter (mm)	24	0.58 ± 0.05	0.61 ± 0.04	0.63 ± 0.10
Femoral SR_{AUC} (s, 10^4)	24	15.8 ± 8.0	16.9 ± 9.0	111. ± 3.9
Brachial FMD%	24	6.60 ± 2.48	6.39 ± 2.96	6.50 ± 2.94
Brachial Baseline Diameter	24	0.34 ± 0.03	0.36 ± 0.04	0.344 ± 0.08
Brachial SR_{AUC} (s, 10^4)	24	10.3 ± 7.6	15.9 ± 828	12.4 ± 8.6
cIMT (mm)	26	0.54 ± 0.04	0.57 ± 0.06	0.59 ± 0.07
Carotid Artery Diameter (mm)	26	6.35 ± 0.39	6.02 ± 0.24	6.38 ± 0.38
Cold Pressor ΔSBP (mmHg)	23	22 ± 11	32 ± 12	25± 14
Cold Pressor ΔDBP (mmHg)	23	17 ± 9	19 ± 9	16 ± 8
Cold Pressor CAR (%)	21	3.54 ± 4.48	5.20 ± 1.46	0.81 ± 7.47

MCAv – middle cerebral artery blood flow velocity; MAP – mean arterial pressure; CVCi – cerebrovascular conductance index; SBP – systolic blood pressure; DBP – diastolic blood pressure; FMD – flow-mediated dilation; SR_{AUC} – shear rate area-under-the-curve; cIMT – carotid intima-media thickness; CAR – carotid artery reactivity.

* Indicates a significant difference compared to LoSIT ($P < 0.05$).

† Indicates a significant difference compared to MidSIT ($P < 0.05$).

2.4 Discussion

The primary aim of the study was to determine whether increased SB is associated with increased cardiovascular risk in highly active individuals. The main findings show that in active adults, higher SB was positively associated with BF%, hip circumference and inversely associated with VO_{2peak}, but was not associated with markers of vascular health and autonomic function including endothelial function, PWV, cIMT, MCAv and CPT responses. These findings seem to imply that engaging in more than the PA guidelines has a protective effect for these vascular health markers, regardless of time spent in SB, but that in order to maintain body composition and CRF levels, both levels of PA and SB are important. This suggests that the relationships between SB, PA and vascular parameters may not be independent of each other.

All participants exceeded the PA guidelines of 150 minutes of MVPA or 75 minutes of VPA per week (World Health Organisation, 2010), but SB was significantly different across groups with daily sedentary time ranging from ~7 hours/day in LoSIT to ~9 hours/day in HiSIT (Table 4). Though a total of 7 hours/day of SB could be deemed as high, there are no guidelines or agreement to determine a threshold of how much SB is detrimental to health. A meta-analysis showed that >8 hours/day of SB significantly increases the risk of all-cause mortality (Chau et al., 2013a) and recently Bennie et al. (2016) defined the threshold for low levels of SB as <8 hours/day in an investigation of PA levels in over 9000 Australian adults. Combined with the results of the current study, perhaps SB guidelines could now begin to be established.

The HiSIT group fit with the "active couch potato" phenomenon, as described previously, of which the hypothesis suggests that in those who adhere to the guidelines, greater SB is still associated with augmented cardiovascular risk (Owen et al., 2010). Our findings partly support this hypothesis, as HiSIT had greater BF%,

larger hip circumference and diminished VO_{2peak} compared to LoSIT (Table 5), all of which impose CVD risk.

Recently, research has demonstrated a significant association between objectively measured daily SB and waist circumference, BP, plasma glucose, triglycerides and cholesterol in individuals who fail to meet the government PA guidelines, but this metabolic risk is mitigated when the guidelines are met (Rao et al., 2016). This evidence exemplifies the beneficial role of PA adhering to guidelines to maintain some specific markers of cardiometabolic health. However the vascular data in the current study did not appear to be related to SB, but further research is needed to determine the relationship between SB and vascular parameters in an inactive population.

2.4.1 Sedentary Behaviour and Body Composition

Findings of the present study demonstrate that SB has a significant association with hip circumference and BF%, irrespective of engagement in high levels of PA, and after adjustment for age and sex (Table 5). However, no association was observed between SB and BMI and waist circumference. Interestingly, it is noteworthy that the HiSIT group would be classed as being overweight according to their BMI above 25kg/m^2 (World Health Organization, 1997), whilst LoSIT and MidSIT are considered healthy weight (18.5-24.9kg/m^2). Despite being statistically insignificant, these results could be clinically meaningful and have important public health implications.

Hip circumference is inversely related with mortality, irrespective of PA (Ostergaard et al., 2013), however limited evidence is available regarding SB and hip circumference, as waist circumference appears to be the preferred and most common measure of body composition. In contrast to the study findings, concurrent evidence reports an

inverse relationship between SB and waist circumference (Chau et al., 2014; Staiano et al., 2014), which is commonly argued to be a stronger predictor of CVD risk (Golubic et al., 2015) as visceral fat is known to be associated with CVD (Mahabadi et al., 2009).

In line with the current findings, several studies have reported an inverse relationship between SB and BF% (Kyle et al., 2001; Shuval et al., 2014; Tucker & Peterson, 2003), even in an active population (Salmon et al., 2000; Sugiyama et al., 2008; Stamatakis et al., 2009). Furthermore, in an active population of Australian adults, Healy et al. (2008b) found a significant detrimental dose-response relationship between TV viewing time and waist circumference. Underlying causes of these relationships could be mechanistic or behavioural, in that SB and PA may instigate different metabolic pathways or individuals may engage in unhealthy snacking behaviours whilst sitting which increase caloric intake. This implies that perhaps the type of SB is relevant when investigating relationships between SB and cardiovascular or metabolic parameters. In summary, it appears that SB has an independent effect on BF%, and even high MVPA cannot offset it.

Golubic et al. (2015) recently conducted a longitudinal 7-year follow up study in the ProActive trial cohort study (231 participants). MVPA and SB were objectively measured, and indices of total- (weight, fat mass and BF%) and abdominal (waist circumference) body fat were assessed at baseline, 1-year and 7-year follow up. Interestingly, the authors found that MVPA was inversely related to all indices of body fat, whilst SB was positively associated with all indices except waist circumference. This implies that the relationship between PA, SB and body fat is bidirectional and independent. Similarly, Du et al. (2013) found that high amounts of self-reported SB was independently associated with higher BF%, and each additional 90 minutes/day of SB was associated with a 0.19kg/m^2 increase in BMI, a 0.57cm larger waist

circumference and 0.44% increase in BF%. In line with our data, the above evidence clearly demonstrates that PA and SB both have subsequent effect on body composition.

2.4.2 Sedentary Behaviour and Cardiorespiratory Fitness

CRF is a known predictor of CVD incidence and mortality (Thorp et al., 2011), and, poor CRF is generally associated with a sedentary lifestyle and physical inactivity (Minder et al., 2014). In line with previous findings (Shuval et al. (2014), the data from the current study indicates that SB was significantly associated with VO_{2peak} (Table 5), however the current results also indicate, for the first time, that there is an inverse association between SB and CRF even in very highly active individuals. The underlying mechanisms linking SB and fitness are unclear, in extreme cases of SB, i.e., bed rest, an increased resting- and maximal heart rate and the compromised stroke volume accompanying this seems to be a potential mechanism for the reduction in VO_{2peak} (Convertino, 1997), through the reduced distribution of oxygen via red blood cells. However, the physiological implications may differ between supine and sitting postures and bed rest is an extreme stimulus. Furthermore, VO_{2peak} has been found to be inversely related to metabolic parameters such as blood glucose and insulin, BP and triglycerides (Lakka et al., 2003), so there could be a metabolic pathway involved in the decrease in CRF related to SB.

In contrast to the present findings, high SB in adolescent females was related with lower fitness among people who did not meet PA guidelines, whereas no association was found when guidelines were met (Martinez-Gomez et al., 2011), suggesting that age could have an impact on the relationship between SB and CRF. Similarly, Eriksen

et al. (2015) reported an inverse relationship between SB and fitness in sedentary participants as well as those engaging in predominantly LPA, but no association was observed between SB and fitness in subjects who engage in mostly MPA and VPA. These results imply that engaging in MPA and VPA offsets the negative effect of SB on CRF; this opposes the findings of the current study which suggest this is not the case.

2.4.3 Sedentary Behaviour and Vascular Structure and Function

2.4.3.1 Carotid Intima-Media Thickness

Reference values for cIMT exist for a healthy population of Uruguayans (Farro et al., 2012). The authors reported normal cIMT values from 0.39 – 0.66mm corresponding to age range of the current study (18-50 years), and the tertile and sex-related mean values fall within this range. There were no significant differences in cIMT between the groups, though there appears to be a trend for higher cIMT as sedentary time increases (Table 5). However, the failure to reach significance could be attributed to the sample size and therefore statistical power of the current study, as opposed to population-based studies. Furthermore, given that all participants fall within the normal cIMT range according to the above reference values, this may be evidence that PA is protective of cIMT.

Compelling evidence shows a negative effect of SB on cIMT in healthy populations (Diaz et al., 2016; Garcia-Hermoso et al., 2015; Kozakova et al., 2010). Diaz et al. (2016) used TV viewing as a surrogate marker for SB, and found that longer TV viewing (>4 hours a day) was associated with greater cIMT in an African American population. However, one must be aware of ethnic differences in cardiovascular health,

as black people have shown to have thicker cIMT (D'Agostino et al., 1996), thus tend to have a higher prevalence of CVD risk (Manolio et al., 2008). Furthermore, Parsons et al. (2016) investigated age-related relationships between SB and cIMT in men aged 78 years from the British Regional Heart Study. The authors found that all PA, irrespective of intensity and duration, and lower SB were beneficially associated with cIMT. Interestingly, the authors reported that a decrease of 30 minutes of daily sedentary time was associated with a 0.0061mm lower cIMT. From this, it is evident that SB is a contributing factor to the development of atherosclerosis, but the above studies have used populations who are at increased CVD risk so they may observe different relationships with SB, PA and cIMT than in the current study.

2.4.3.2 Endothelial Function

Findings of the present study showed no differences between groups in brachial and femoral FMD responses (Table 5), which suggests that when the PA guidelines are met, SB is not associated with diminished conduit vascular function. Although this is in line with previous data in children which also found no association between SB and endothelial function (Hopkins et al., 2012), the current findings may be surprising given that numerous recent studies have indicated that FMD declines following 3 hours of sitting (Thosar et al., 2014; Thosar et al., 2015a), and that FMD can show potentially clinically meaningful improvements when sitting time is reduced over 8 weeks (Graves et al., 2015). However numerous studies also show that if blood flow and shear stress is maintained, by using short bouts of activity (McManus et al., 2015; Restaino et al., 2015; Thosar et al., 2015a) or as recently discovered, by fidgeting (Morishima et al., 2016), then FMD is maintained. One issue with the FMD test however, is that results (as a percentage change) cannot be contextualised as reference values currently do

not exist, having said this, FMD data across all tertiles is similar to that reported previously in healthy individuals (Juonala et al., 2008).

A mechanistic explanation of the study findings may be related to oxidative stress, which is generated by an imbalance in pro- and anti-oxidant molecules in the blood (Lessiani et al., 2016). Oxidative stress is known to induce endothelial dysfunction (Cai & Harrison, 2000) and is also associated with the development of atherosclerosis (Witztum, 1994). In mice, a sedentary lifestyle is related to increased oxidative stress, which in turn attenuates endothelial function (Laufs et al., 2005). Furthermore, Thosar et al. (2015b) found that ingesting vitamin C (an antioxidant) prevented the decline in femoral FMD observed following 3 hours of continuous sitting. With these findings in mind, the current data indicating no difference between groups for FMD may be surprising, as it would be expected that those that engage in excessive sitting to incur greater oxidative stress. However, PA has been associated with enhanced lipid peroxidation and antioxidant (superoxide dismutase; SOD and glutathione peroxidase; GSH-Px) enzyme levels (Covas et al., 2002), which together provide protection against free radical production via a scavenging system. Aerobic exercise stimulates the free radical scavenging system, including SOD and GSH-Px (Schuler et al., 2013), therefore decreasing oxidative stress by increasing the efficiency of the antioxidant system. Furthermore, PA has been discovered to result in increased eNOS mRNA in mice (Laufs et al., 2005), and similar responses occur with augmented shear stress in vivo and in cell culture models (Noris et al., 1995; Uematsu et al., 1995). From this increase in eNOS mRNA, bioavailability of NO is improved, leading to enhanced antioxidant defence and reduction in pro-oxidant molecule expression (Gliemann et al., 2014). Together, the above evidence implies that in the presence of increased SB,

and therefore oxidative stress, highly active individuals may benefit from an upregulation of the free radical scavenging system which protects endothelial function.

2.4.3.3 Arterial Stiffness

Results of the current study demonstrated no relationship between SB and PWV and all participants' PWV values were around what is considered normal (Table 5; Muller et al. (2013)). Conversely however, recent research has established a positive association between SB and stiffness (Horta et al., 2015), with individuals surpassing the PA guidelines having lower PWV. Similarly, Quan et al. (2014b) reported a relationship between SB and stiffness, independent of PA. The authors had previously found an association between PA and arterial stiffness, which appeared to be mediated by resting heart rate (Quan et al., 2014a), however the association of SB with arterial stiffness was not mediated by resting heart rate, BF% or metabolic parameters, therefore the mechanisms remain unclear. The above studies comprised of a population of over 2000 and 1000 participants respectively, which could explain why the authors observed a negative effect of SB on stiffness whilst no effect was observed in the current study with lower participant numbers.

Arterial stiffness is predominantly determined by age and BP (AlGhatrif et al., 2013) and is reported to be negatively associated with CBF (Jaruchart et al., 2016). As no differences were observed in age, BP and MCAv across the groups (Table 5), this could explain why no difference in PWV was observed. However, previous research has demonstrated a relationship between stiffness and BF% (Mac Ananey et al., 2015) as well as CRF (Boreham et al., 2004; Quan et al., 2014a). Mac Ananey et al. (2015) found a significant correlation between objectively measured SB, PWV and BF% in

healthy individuals. This suggested relationship between BF% and PWV was not evident in the present study as no difference was observed in PWV between groups, whilst there was a significant difference in BF%.

Stiffening of the artery occurs through changes in structural properties and the balance of collagen and elastin (Zieman et al., 2005). Presence of inflammatory markers such as CRP, inter-leukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) and high BP can lead to overproduction of collagen and a reduction in elastin molecules, which contribute to arterial stiffening (McEniery & Wilkinson, 2005). As all of our participants were normotensive, and speculated that their antioxidant capacity is good, this may prevent changes in stiffness.

2.4.3.4 Cerebral Blood Flow

Findings from the present study suggest there is no association between sedentary time and MCAv (Table 5) in an already active population. However, although scant, findings from previous research have contrasted the results of the current study. Ainslie et al. (2008) were the first authors to discover that CBF was lower in inactive subjects, compared to those engaging in regular aerobic exercise. Similarly, MCAv was found to be attenuated in sedentary (defined by no formal recreational activity reported) compared to trained individuals irrespective of age (Bailey et al., 2013). These studies suggest that PA is important, and given that participants in the current study were all active, this may protect MCAv. This is a novel finding, in a highly active population, SB is not associated with reduced CBF. However the relationship between moderately active and inactive participants CBF and SB is still unknown.

2.4.4 Sedentary Behaviour and Autonomic Function

No differences were observed between groups for BP response and CAR% during CPT (Table 5). Currently however, further evidence is lacking regarding the relationship between SB and autonomic function as measured by the CPT, nevertheless some insight into PA effects exist. A combination of low PA, high baseline SBP and BMI has previously been found to be associated with greater BP response to CPT (Zhang et al., 2013). On the other hand, as previously discussed, Bond et al. (2001) found similar BP CPT responses between active and inactive subjects, suggesting that PA status may not contribute to the BP response to the CPT. However, the authors did not define subjects as active or inactive by the PA guidelines; the authors used questionnaire responses and defined active and inactive as performing aerobic exercise for 20 - 30 minutes three times per week and less than one day a week respectively. Therefore, it remains unclear whether meeting the PA guidelines would have an effect on BP response.

Carotid artery reactivity is not a novel research method, but is seldom utilised to assess CPT responsiveness. In healthy individuals, the “normal” response of the carotid artery during the CPT is dilation, whereas constriction is associated with the presence of coronary risk factors (Rubenfire et al., 2000). The raw data from the present study indicate that only 5 out of 21 participants experienced vasoconstriction of the carotid artery, of which 2 participants were in LoSIT group and 3 in HiSIT. Interestingly, post-hoc analysis revealed that all participants who demonstrated carotid artery vasoconstriction in response to the CPT had a brachial FMD response <4%. These results suggest that FMD <4% causes a CAR% constriction response. Furthermore, Rubenfire et al. (2000) found a correlation between CAR% and cIMT, which suggests

that wall thickness contributes to endothelial function and stiffness, limiting the ability of the artery to dilate.

2.4.5 Sex Differences

Unsurprisingly, sex-specific differences were observed in waist:hip ratio, BF%, VO_{2peak} and MCAv, whereby females demonstrated lower waist:hip ratio and VO_{2peak} and greater BF% and MCAv than males (Table 2). There were no differences in PA and sedentary time between sexes (Table 3), suggesting other factors are the underlying cause of the observed differences. The female sex hormone oestrogen is associated with distribution of- and total BF% (Karastergiou et al., 2012), and MCAv (Shamma et al., 1992). Females tend to store fat peripherally such as in the limbs and hips, whereas males store fat centrally (Geer & Shen, 2009), and this determines the difference in waist:hip ratio as observed in the present study. MCAv has also been reported to be greater in female children (Vavilala et al., 2005) and adults alike (Bakker et al., 2004). However, there does not appear to be any association between oestrogen and fitness (Campbell et al., 2005), though it has been hypothesised that sex differences in fitness are likely attributed to a difference in muscle mass and haemoglobin concentration (Armstrong & Welsman, 1994) between males and females. Haemoglobin is important in the transport of oxygen in the blood, therefore increased concentration would improve oxygen transport and delivery during exercise, leading to enhanced VO_{2peak}.

2.4.6 Strengths and Limitations

Firstly, a major strength of the current study is that PA was measured objectively via accelerometry, which eliminates social desirability bias associated with self-report measures. Furthermore, the combination of ActiGraph and ActivPAL enhanced the reliability of sedentary data, because the ActivPAL is most reliable method for measuring SB (Grant et al., 2006) as the device provides posture data to distinguish between sitting and standing behaviour, which is important in research of this nature. Variation in PA was accounted for by all participants meeting the guidelines, and employing this gives an insight into whether PA and SB are independent of each other. Moreover, the current study involved collecting a broad range of cardiovascular risk factor data, using gold standard measures, to assess and explore various mechanistic pathways, however blood samples to assess inflammatory markers would have provided further confirmative evidence regarding oxidative stress.

One limitation of the current study is sample size, so the data has low statistical power, which means that differences may have been missed between some variables. If the sample size was greater, more data may have reached statistical significance. Also, there was not an even distribution of age, as the majority of participants were aged 18 – 35 years, and only 3 out of 26 subjects were over 40 years old. However all participants were defined as active by meeting the PA guidelines, and data indicates that maintenance of activity with aging prevents decline in vascular function (Black et al., 2009). Additionally, the study could have been strengthened by the inclusion of an inactive reference group to compare the effect of SB on cardiovascular biomarkers. Finally, methodological issues are regularly raised regarding the use of bioelectrical impedance to assess BF% as opposed to the gold standard dual energy x-ray

absorptiometry. This simple and quick method has been shown to be valid in estimating total body water, and with normal hydration, fat free mass (Ellis et al., 1999).

2.4.7 Implications and Future Directions

The current research has potentially important public health implications, as engaging in high amounts of SB whilst meeting the PA guidelines (an active couch potato) is detrimental to CRF and body composition, both of which are well recognised CVD risk factors. These results support the case for SB guidelines to be introduced in addition to the current PA recommendations. In addition, our findings also indicate that body composition and fitness appear to have independent relationships with SB and PA. However independent relationships do not appear to exist between SB and markers of vascular and autonomic health, suggesting that high levels of activity can offset any detrimental effects of SB.

Future research should firstly endeavour to investigate the relationships between the CVD markers and SB across a range of PA levels, and also investigate the mechanistic pathways relating SB to cardiovascular biomarkers, including inflammatory markers and blood metabolites to gain a better understanding of oxidative stress and cardiometabolic outcomes and their interaction. Current research considering CBF and SB is scarce, and exploration of functional measures of the cerebrovasculature such as cerebrovascular reactivity and autoregulation is a novel area to explore in the future. The SB data of the present study shows that workplace sedentary time is the biggest contributor to total sedentary time

Furthermore, an investigation into sedentary bouts and activity breaks, similar to the "breaker vs prolonger" first established by (Healy et al., 2008a), is required to establish whether the distribution of sedentary time rather than total SB is related to vascular structure and function and autonomic function measures.

2.4.8 Conclusion

In conclusion, in an active population of healthy adults, greater time spent in SB appears to be negatively associated with CRF and body composition including hip circumference and BF%. Engaging in >150 minutes of MVPA or >75 minutes of VPA per week appears to be protective of the vasculature and cardiovascular autoregulation. Further research is required to establish potential underlying mechanisms of this relationship.

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