Associations between physical activity and sedentary time with endothelial function, arterial stiffness, arterial elasticity, and clustered cardiometabolic risk in children: The ALSPAC Study

Submitted by Kate Marie Sansum, to the University of Exeter as a thesis for the degree of Masters by Research in Sport and Health Sciences

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature……

Abstract

***Introduction*:** Evidence for associations between physical activity (PA) or sedentary time (ST) and vascular health in paediatric populations is of low quality due to the predominance of self-report measures of PA time and intensities, small sample sizes, and a lack of control of confounding variables. This thesis examined associations between device-derived PA and ST with vascular outcomes and a clustered cardiometabolic risk (CMR) score in a population sample, with adjustment for covariates. ***Methods*:** Cross-sectional analysis of 4,277 children (2,226 girls) aged 10.6 ± 0.2 y from the Avon Longitudinal Study of Parents and Children. Clustered CMR was measured at age 9 y. Vascular outcomes (flow mediated dilation, distensibility coefficient, and pulse wave velocity) were measured age 10 y. Light and moderate to vigorous PA (MVPA) and ST were measured via accelerometers at age 11 y. Multiple linear regression analyses were used to examine associations between exposures and outcomes, with PA and ST entered as compositional exposure variables and non-compositional variables (min·day^{-1}). ***Results*:** Neither light PA, MVPA or ST were significantly associated with any of the vascular outcomes in the adjusted compositional and non-compositional models. The proportion of time spent in MVPA and ST (relative to the remaining activity behaviours) were inversely (b=-0.126; *P*=0.001) and positively (b=0.136; *P*=0.016) associated with CMR in the whole group analysis, respectively. MVPA was negatively associated with CMR in both boys (b=-0.144; *P*=0.011) and girls (b=-0.110; *P*=0.032), but only girls had a positive association between ST and CMR (b=0.199; *P*=0.005). In the non-compositional models, MVPA was inversely associated with CMR in the whole group analysis (b=-0.002; *P*=0.012). In the girls, ST was positively (b=0.001; *P*=0.035), and LPA was inversely (b=-0.001; *P*=0.035) associated with CMR.

***Conclusion*:** Longer exposure to CMR factors during adolescence may be needed to establish relationships between PA and ST with vascular outcomes. These findings support interventions that promote MVPA and minimise ST for reducing CMR in children. Prospective studies are required to understand the causal directions.

Key words: flow mediated dilation, pulse wave velocity, paediatrics, compositional data analysis

“Rivers know this: There is no hurry.

We shall get there some day”

A. A. Milne

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Definitions and abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
aIMT	Aortic intima media thickness
aPHV	Age in years from peak height velocity
BMI	Body mass index
BMI-SDS	Body mass index standard deviation score
cIMT	Carotid intima media thickness
CI	Confidence interval
CMR	Cardiometabolic risk
CoDA	Compositional data analysis
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DC	Distensibility coefficient
DEXA	dual energy X-ray emission absorptiometry
FMD	Flow mediated dilation
HDL	High density lipoprotein
Ilr	Isometric log ratio
LDL	Low density lipoprotein
LPA	Light physical activity
MAP	Mean arterial pressure
MPA	Moderate physical activity
MVPA	Moderate to vigorous physical activity
PA	Physical activity
PWC_{170}	Peak work capacity at 170 beats per minute

PWV	Pulse wave velocity
SB	Sedentary behaviour
SBP	Systolic blood pressure
SD	Standard deviation
SPSS	Statistical packages for social sciences
ST	Sedentary time
TAG	Triglyceride
VPA	Vigorous physical activity

Chapter 1

Introduction

Cardiovascular diseases (CVD), such as myocardial infarction and strokes, are the leading cause of non-communicable diseases worldwide (World Health Organization, 2020). In the United Kingdom (UK), CVDs are responsible for 44,000 premature deaths per year (i.e. <75 y old) (British Heart Foundation, 2020). Although mortality rates from CVD have fallen in recent decades, in 2017 it was estimated that they resulted in 2,202 years of healthy life lost (age-standardised rate per 100,000 inhabitants) in the UK (British Heart Foundation, 2019). Heart and circulatory system diseases elicit yearly healthcare costs of ~£9 billion in the UK alone (British Heart Foundation, 2020). Thus, strategies to reduce the burden of CVD warrant investigation.

Atherosclerosis precedes the most prevalent forms of CVD (McGill et al., 2000a) with impairments in endothelial function occurring before the structural alterations (e.g. fatty streaks and fibrous plaques) present (Ross, 1993, Juonala et al., 2004). Although the clinical manifestations of CVD present in later life, autopsy evidence demonstrates the presence of fatty streaks in children and adolescents from as early as 1 y old (Klotz and Manning, 1912, Stary, 1989). Furthermore, the prevalence of fatty streak development in key arteries in youth is proportional to CVD risk factor exposure in youth (Berenson et al., 1998). Exposure to CVD risk factors in childhood and adolescence is also associated with changes to the vascular structure in adulthood (Raitakari et al., 2003). A risk factor is defined as a biological characteristic that precedes an outcome of a disease, predicts the outcome, and is directly in the causal path of the disease (Balagopal et al., 2011). “Traditional”, well-established, CVD risk factors include hypertension, dyslipidaemia and obesity (Kavey et al., 2003). However, individuals can present with structural changes to the artery without exposure to traditional risk factors

(Berenson et al., 1998). Consequently, research has examined “novel” risk factors of CVD to explain the changes in vascular structure and function in the absence of traditional risk factor exposure (Balagopal et al., 2011). Directly measuring endothelial function (a barometer of vascular health (Vita and Keaney, 2002)) rather than measuring variables that influence vascular health has gained popularity in recent decades. Vascular function is an important measure because asymptomatic children and adolescents with clustered CVD risk factors have impaired endothelial function compared control participants (Celermajer et al., 1992). Additionally, endothelial function protects against unfavourable structural changes to the vessel (Juonala et al., 2004). Arterial stiffness provides information surrounding both the function and structure of the vessel, and children with hypercholesterolemia present with increased arterial stiffness compared to healthy controls (Riggio et al., 2010). Therefore, interventions that target preserving vascular function and limiting detrimental changes to the vascular structure during childhood play a vital role in the primary prevention of CVD (Juonala et al., 2004).-The American Heart Association reported that public policy and community efforts focusing on the prevention of CVD such as encouraging physical activity (PA) and improving diet, are not only cost-effective, but are also effective at preventing cardiac events (Weintraub et al., 2011). Consequently, research that targets the primary prevention of CVD, especially in paediatric populations, is essential.

Physical activity (PA) refers to any movement produced by skeletal muscles that results in energy expenditure (Caspersen et al., 1985). Sedentary behaviour (SB) is defined as an energy expenditure ≤1.5 metabolic equivalents when either sitting, lying or reclining, and sedentary time (ST) refers to the

amount of time spent performing SBs such as watching television, reading, travel and working (Tremblay et al., 2017). The recently updated UK PA guidelines provide age specific details for duration and intensity, with children and adolescents (aged 5-18 years) recommended to spend at least an average of 60 minutes per day engaged in moderate to vigorous PA (MVPA) across the week (UK Chief Medical Officers', 2019). The guidelines also advise children and adolescents to minimise ST and break up prolonged periods of inactivity with at least light physical activity (LPA). It is possible to meet the PA guidelines but also be classed as highly sedentary. For example, a child can perform 60 minutes of MVPA per day to meet the PA guidelines but spend the majority of their remaining waking hours sedentary, rather than also engaging in a large amount of LPA. This is because MVPA typically accounts for a very small proportion of waking time, leaving many hours remaining to be split between ST and LPA. For example, if child meets the PA guidelines of 60 minutes of MVPA per day, this only accounts for ~6% of the waking day if the child is awake for 16 hours and leaves 15 hours to be split between LPA and ST. The influence of each activity behaviour (ST, LPA and MVPA) on health outcomes needs to be examined while accounting for the influence from the other activity behaviours. This is because without including the other activity behaviours, the "true" association between an activity behaviour (e.g. ST) and a health outcome (e.g. a CVD risk factor) may be masked by the influence of variance in the remaining activity behaviours (e.g. LPA and MVPA) which has not been accounted for in the analytical model. For example, the significant positive association between ST and insulin concentration in a sample of 20,871 children and adolescents was attenuated to null after adjustment for time spent in MVPA (Ekelund et al., 2012). However, there is still likely residual confounding variance influencing

this association from the differences in LPA between participants that was not accounted for in the model.

The evidence informing the PA and ST guidelines is primarily based upon epidemiological evidence reporting associations between PA, SBs and traditional CVD risk factors (Sardinha et al., 2008, Ekelund et al., 2012, Vaisto et al., 2014, Barker et al., 2018). An unfavourable association has been reported between traditional CVD risk factors and arterial structural and function outcomes in children and adolescents (Berenson et al., 1998, Fernhall and Agiovlasitis, 2008), yet few studies have comprehensively explored the association between precise, device-based measures of PA and ST with direct measures of arterial structure and function. Contemporary reviews have shown that the existing evidence base for associations between ST or PA and vascular outcomes (Baumgartner et al., 2020, Konigstein et al., 2020) is currently of low quality, with small sample sizes (*n*=54-1729), a lack of covariates in the analytical models and the use of self-report methods to measure PA and ST. Therefore, the associations presented in the literature may contain errors from the absence of important covariates, and from less precise measurement of the predictor variables (PA and ST) through the use of self-report measures.

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a large prospective birth cohort study from Bristol, UK (Golding et al., 2001). It has data on over 15,000 participants, including device-derived PA and ST data, assessment of traditional CVD risk factors and direct measures of vascular function and structure (endothelial function, arterial stiffness, and arterial elasticity) during childhood and adolescence, and into early adulthood. This

means the ALSPAC dataset is uniquely suited to address the aforementioned limitations within the current literature. Therefore, this thesis aims to comprehensively examine the cross-sectional associations between device-derived ST and PA with endothelial function, arterial stiffness, and elasticity and clustered cardiometabolic risk (CMR) in a population sample of contemporary children using the ALSPAC dataset.

Chapter 2

Literature review

This chapter discusses the research that established that CVD begins in childhood. It also presents a critical discussion of the literature that has investigated the relationships between PA and ST with a clustered CMR score, vascular function and structure in paediatric populations.

2.1 PAEDIATRIC ORIGINS OF CARDIOVASCULAR DISEASE

2.1.1 The atherosclerotic process

The presence of sub-clinical markers of CVD in youth was initially documented from autopsies in the early 20th century (Klotz and Manning, 1912). Later evidence showed this phenomenon was commonplace with 65% of ostensibly healthy 12-14 y olds presenting with fatty streaks in their coronary arteries, and 8% of the sample had more advanced atherosclerotic lesions present in post mortem examinations following non-natural deaths (Stary, 1989).

Atherosclerosis precedes the most prevalent forms of CVD (Figure 2.1) whereby low-density lipoproteins, chylomicron remnants and white blood cells penetrate the endothelium (single layer of cells that make up the interior wall of the artery) and form foam cells (Stocker and Keaney, 2004). A fatty streak forms that can later develop into a fibrous plaque (Katz et al., 1976). This may then develop into an advanced complicated lesion with the continued infiltration of lipids, smooth muscle cell proliferation and migration, and calcium accumulation (Ross et al., 1984, Stary et al., 1995), narrowing the lumen of the artery. If this lesion ruptures, releasing the lipid-rich plaque into the circulation, a cardiovascular event may occur. The clot may directly cause an ischemic event, or it can promote an aneurysm.

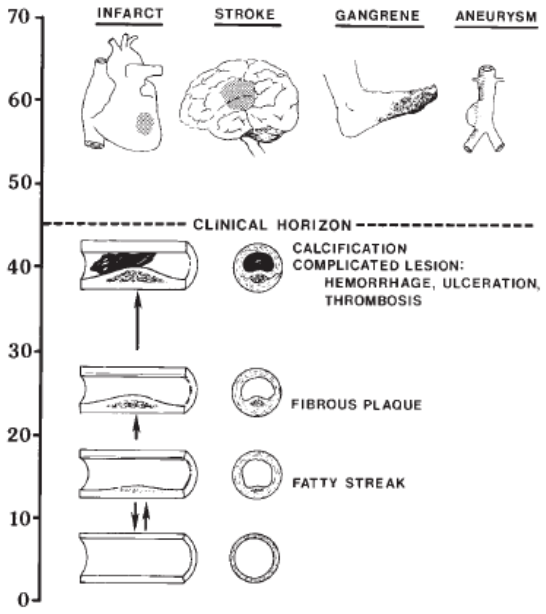

Figure 2.1 - The progression of atherosclerosis. Reproduced with permission from McGill et al. (2000).

Impairments in endothelial function occur before the structural alterations present (Ross, 1993, Juonala et al., 2004) and endothelial dysfunction is characterised by a reduction in the bioavailability in endothelium derived nitric oxide (Stocker and Keaney, 2004). The endothelium plays a key role in regulating vascular tone by secreting vasodilators such as nitric oxide (Ignarro, 1989) and vasoconstrictors such as angiotensin II (Dzau, 1988). Transient impairments in endothelial function allow the infiltration of low-density lipoproteins, chylomicron remnants and cellular debris into the arterial wall, and thus are a sentinel event in the progression of atherosclerosis. Additionally, the maintenance of endothelial function provides protection against structural changes (Juonala et al., 2004), making endothelial function an attractive target in the primary prevention of CVD. Furthermore, endothelium-derived nitric oxide is anti-atherogenic, so promoting endothelial function may inhibit the formation of new lesions (Cooke and Tsao, 1994).

2.1.2 Cardiovascular disease risk factors

The Bogalusa Heart Study provided evidence that the prevalence of fatty streaks and fibrous plaques in both the aorta and coronary arteries in 93 young individuals (2-38 y old) who died from traumatic injuries was proportional to CVD risk factor exposure (Berenson et al., 1998). These deleterious associations between CVD risk factor exposures (e.g. hypertension, poor glucose tolerance and non-high-density lipoprotein (HDL) cholesterol concentration) and structural changes have also been replicated in the Muscatine Study (Mahoney et al., 1991) and the Pathological Determinants in Youth study (McGill et al., 1995, McGill et al., 1997, McGill et al., 1998, McGill et al., 2000b). However, although the sample sizes in most of these studies were relatively large (*n*=820-2,403), the samples were heterogeneous in nature, especially in terms of age, which could be altering the strength of the observed associations. The wide age ranges encompassing childhood to adulthood limit the extent to which conclusions can be drawn about the magnitude of the associations in the individual age groups. Specifically, the inclusion of adults in the child and adolescent samples in some studies such as Berenson et al. (1998), means that the inference of paediatric origins of CVD could be considered questionable as the adult data may be driving the associations. Therefore, further investigations in more homogenous samples are required to provide clarity on the magnitude of the associations in specific age groups. Additionally, the studies were limited in the outcomes that they were able to include, which resulted in the absence of examination of novel risk factors such as endothelial function.

The CVD risk factor profiles (the number of risk factors and composite risk factor scores) in childhood and adolescence (3-18 y old) have been shown to predict adult carotid intima media thickness (cIMT), measured 21 y later (Raitakari et al., 2003, Laitinen et al., 2012); a clinically important marker or atherosclerotic progression (Touboul et al., 2004). In addition, the risk factor profiles in youth are stronger predictors of adult cIMT than the adult risk factor profiles. The evidence suggests that the changes to the artery wall structure begins early in life. In fact, the individuals with the worst CVD profile in adolescence (12-18 y at baseline) had the amount of thickening of the cIMT present when in adulthood (21 y later) that would be expected if they were an additional 12 y older (Laitinen et al., 2012). However, recent data have suggested that some of the structural changes to the vascular system observed throughout childhood and adolescence are adaptations to growth and development rather than pathological damage per se as it appears to be predicted by lean body mass (Sletner et al., 2018) and it tracks with the changes in lean body mass (Chiesa et al., 2019). Therefore, it is likely that not all of the changes to the artery exhibited during early life have clinical relevance (Agbaje et al., 2021a). Furthermore, some of the associations between CVD risk factors and vascular outcomes may be bi-directional (i.e. changes to vascular outcomes predict changes in CVD risk factors). For example, data from the ALSPAC study found carotid-femoral PWV was bi-directionally associated with adiposity between 17 and 25 y and carotid-femoral PWV at 17 y predicted adiposity and hypertension at 25 y (Agbaje et al., 2022).

2.1.3 Tracking of cardiovascular disease risk factors in childhood and their translation into adulthood

Tracking refers to how stable a risk factor is over time and whether early measurements of a risk factor can predict the risk factor later in life (Twisk et al., 1997). A tracking coefficient is a longitudinal correlation coefficient between repeated measured of a risk factor (Twisk, 2003). Although it can range from -1 to +1, it is typically between 0 and +1 due to the assumption that the correlation between the repeated observations is positive. A higher value demonstrates stronger stability over time. Studying whether risk factors track is beneficial because individuals who are at risk can be identified early and targeted with interventions to try and reduce that risk. There is variability in the tracking of individual CVD risk factors from childhood and adolescence into adulthood (Mahoney et al., 1991, Porkka et al., 1991, Twisk et al., 1997). Blood pressure appears to track relatively weakly with systolic blood pressure (SBP) tracking coefficients ranging from 0.21 to 0.39 and diastolic blood pressure (DBP) from -0.01 to 0.50 between school-age children (7-18 y old) and young adulthood (20-30 y old) (Mahoney et al., 1991). However, the tracking of serum lipid variables is better with the three-y tracking coefficient for cholesterol for boys at 0.73 and 0.65 for girls (Porkka et al., 1991). The presence of multiple risk factors known as “clustering” can occur in childhood and adolescence (Andersen et al., 2003). The tracking of a cluster of CVD risk factors from adolescents to adulthood is 6 times stronger than that of individual risk factors (Andersen et al., 2004). The tracking coefficients of single risk factors were between 0.20 and 0.72, whereas the tracking coefficient for the number of risk factors an individual had was 0.42 because the cluster contains some elements that track well and others that do not track so well. A clustered cardiovascular risk factor score can be defined as

the mean of the z scores of systolic blood pressure, triglyceride concentration, total cholesterol/HDL ratio, insulin resistance, sum of four skinfolds, and aerobic fitness (Andersen et al., 2006). However, there is not a universal clustered risk score used in the paediatric literature (Stavnsbo et al., 2018). Additionally, a clustered cardiovascular risk score is also known as a CMR score.

2.2.4 The effect of risk factor exposure reduction on cardiovascular disease risk

Many CVD risk factors are modifiable through diet and exercise/physical activity interventions to reduce disease risk (Kavey et al., 2003). Although existing data does not demonstrate that modifying risk factor exposure reverses or reduces fatty streak development, research has found body mass index (BMI) in childhood and adolescence (3-18 y old) is positively associated with cIMT and inversely associated with arterial elasticity in adulthood (21 y later) (Raitakari et al., 2005). Additional analyses showed that these associations were partially explained by the tracking of BMI over time, highlighting the need to maintain a healthy BMI throughout the transition between childhood and adulthood to decrease CVD risk. However, recent data showed it is likely to be lean mass via BMI driving the cIMT changes observed, and this may indicate that the changes in cIMT from BMI are not deleterious (Agbaje et al., 2021a). Additionally, a recent paper showed the association between adiposity and arterial stiffness was bi-directional, suggesting that interventions that improve arterial stiffness directly in adolescence may also be required (Agbaje et al., 2022). Furthermore, increased cardiorespiratory fitness at adolescence (~17 y) was associated with a reduced aortic IMT (aIMT; a measure of pre-clinical atherosclerosis in paediatrics (Järvisalo et al., 2001)) but not with cIMT at adolescence (Pahkala et al., 2013). Moreover, increased

cardiorespiratory fitness in adolescence is associated with reduced CVD risk in adulthood including decreased fatness (Eisenmann et al., 2005) and reduced total serum cholesterol (Boreham et al., 2002), and is independent of overweight/obesity (Schmidt et al., 2016).

High cardiorespiratory fitness in adolescence may play an important role but cannot reverse the increased risk of a myocardial infarction or mortality due to obesity (Hogstrom et al., 2014, Högström et al., 2016). In addition, cardiorespiratory fitness at age 13 y (but not leisure time PA measured via questionnaire) is favourably associated with CVD risk factors (but not CVD) at age 15, 25 and 33 y old, but not aged 40 y (Kvaavik et al., 2009). This highlights the importance of considering the influence of PA and cardiorespiratory fitness on CVD risk separately.

2.2 VASCULAR FUNCTION AND STRUCTURE AS NOVEL RISK FACTORS OF CARDIOVASCULAR DISEASE

Since the early autopsy data of the Bogalusa Heart Study (Berenson et al., 1998), the Muscatine Study (Mahoney et al., 1991) and the Pathological Determinants in Youth Study (McGill et al., 1995, McGill et al., 1997, McGill et al., 1998, McGill et al., 2000b), advances in technology in recent decades has facilitated the development and use of non-invasive high-resolution ultrasound techniques to measure indicators of vascular function and structure. For example, these techniques enable the measurement of endothelial function, arterial stiffness, arterial elasticity and cIMT. The use of non-invasive high-resolution ultrasound techniques are especially useful in paediatric populations where it is often considered unethical to use the invasive alternatives.

2.2.1 Endothelial function

The measurement of endothelial function via flow-mediated dilation (FMD) was first pioneered in the early 1990s by Celermajer and colleagues (1992). The measurement of FMD in the periphery (brachial artery) is a surrogate measure of coronary artery endothelial function (r=0.36; *P*=0.01) (Anderson et al., 1995). FMD is the dilation of an artery in response to an increase in blood flow and shear stress. When FMD is performed following standardised procedures (Thijssen et al., 2019), it is assumed from research in adults that FMD in paediatric groups also reflects endothelial-dependent vasodilation (i.e. nitric oxide mediated) rather than endothelial-independent vasodilation (Doshi et al., 2001, Green, 2005). To confirm FMD reflects endothelial-dependent vasodilation, an additional measurement using a nitric oxide donor such as nitroglycerin would be required. Therefore, this thesis considers FMD to be reflective of endothelial-dependent vasodilation. Impairment of FMD in the brachial artery has been shown to strongly relate to the extent of coronary artery disease present in adults with and without coronary artery disease (Neunteufl et al., 1997). Meta-analyses have since supported these strong associations between impaired FMD and future CVD events in adults (Inaba et al., 2010, Xu et al., 2014), leading to it being used as a novel risk factor of CVD. Furthermore, a 1% increase in brachial artery FMD (when confounding risk factors such as age, and either some or all of the traditional Framingham cardiovascular risk factors (D'Agostino et al., 2008) are adjusted for) is beneficial in reducing relative risk of experiencing a future cardiovascular event in adults by 10-13% (Xu et al., 2014, Inaba et al., 2010). Consequently, a small change in FMD can have a large beneficial impact on reducing CVD risk in adults. Additionally, in asymptomatic adults impaired FMD

is also related to traditional CVD risk factors such as hypertension (Felmeden et al., 2003) and low HDL concentration (Li et al., 2000).

Vascular function is also an important measure in paediatric populations because asymptomatic children and adolescents with clustered CVD risk factors show an impairment in endothelial function in both the brachial and superficial femoral arteries compared to control participants (Celermajer et al., 1992). Youth with obesity and diabetes have impaired FMD compared to ostensibly healthy controls (Fernhall and Agiovlasitis, 2008). Additionally, in youth endothelial function is protective against structural changes to the vasculature (Juonala et al., 2004). Therefore, assessing endothelial function is important to identify individuals at risk of CVD and interventions that promote endothelial function may be able to slow the progression of atherosclerosis (Celermajer, 1997). However, a clinically meaningful change in FMD following interventions is currently unknown for paediatric groups.

In youth, cross-sectional data report that there is an age-related decrease in FMD from a mean of 8.72% age 6 y, to 7.11% at age 18 y (Hopkins et al., 2015). Sex differences exist in this age-related decline in FMD, with the lower FMD only being significant in boys and with the decline occurring after puberty in the later adolescent years. However, when baseline diameter is included as a covariate, the sex-differences are diminished. The sex differences in FMD aged 17 and 18 y with females presenting a higher FMD than males (8.31% vs 7.62%) were considered to be driving the differences in sexes between 6-18 y, and therefore, the sex differences between 6-16 y do not appear to be meaningful. Biological sex has also been reported to be a predictor of FMD in data from children from

the ALSPAC study (Donald et al., 2010), with females presenting with a higher FMD than the males.

2.2.2 Arterial stiffness

Arterial stiffness is determined by the elastin to collagen ratio within the arterial wall (Anderson, 2006). It is a measure of structural change, along with arterial compliance and distensibility (Urbina et al., 2009). A major determinant of arterial stiffness is arterial pressure, with increased arterial stiffness found in patients with hypertension (Laurent et al., 1993). Arterial stiffness can be assessed non-invasively including via applanation tonometry to measure arterial waveforms (Bank et al., 1999). From this, a number of parameters can be calculated: compliance, circumferential wall stress, circumferential strain, Young's elastic modulus and pulse wave velocity (PWV).

Increased arterial stiffness is associated with an increased risk of CVD (Oliver and Webb, 2003, Mattace-Raso et al., 2006, van Sloten et al., 2015). It is also now well established that a non-invasive clinical index of arterial stiffness, PWV, is a predictor of cardiovascular events and all-cause mortality in adults (Boutouyrie et al., 2014, Blacher et al., 1998, Laurent et al., 2001, Vlachopoulos et al., 2010). Furthermore, PWV is elevated in adults with CVD risk factors such as older age (Avolio et al., 1983) and diabetes (Lehmann et al., 1992).

The gold standard measure of arterial stiffness is carotid-femoral PWV (Van Bortel et al., 2012) but arterial stiffness can also be measured at alternative sites such as the brachial-ankle (Sugawara and Tanaka, 2015) and carotid-radial PWV (Laurent et al., 2006). However, alternative sites are less widely accepted

because there are questions surrounding the clinical importance of assessing peripheral stiffness (Sugawara and Tanaka, 2015). Nevertheless, a 1 $m \cdot s^{-1}$ increase in brachial-ankle PWV has been associated with a 12% increase in risk of CVD in adults (Vlachopoulos et al., 2010).

Children and adolescents with type I diabetes have a higher PWV compared to healthy controls (Stella et al., 1984, Woolam et al., 1962). Furthermore, children with hypercholesterolemia display impaired PWV compared to healthy controls (Riggio et al., 2010). A recent systematic review reported that obesity was not consistently associated with carotid-femoral PWV (Stoner et al., 2020). Furthermore, the results of the meta-regression showed that carotid-femoral PWV increased by 0.12 $m \cdot s^{-1}$ per y of age. Biological sex is a significant predictor of PWV, with females presenting with a lower PWV than males in childhood (Donald et al., 2010). Age is also a significant predictor of PWV, with older children presenting with a higher PWV than younger children.

2.2.3 Arterial elasticity

Arterial compliance and distensibility are alternative measures of arterial stiffness but they also represent contrasting aspects of both arterial structure and function. Specifically, distensibility measures the elastic properties of an artery from the relative diameter or area change for a pressure increment (O'Rourke et al., 2002). In contrast, compliance is defined as the absolute diameter or area change for a given pressure step at a fixed vessel length, therefore it measures the vessel's ability to respond to a change in blood volume.

Data from the Bogalusa Heart Study identified that arterial elasticity was inversely associated with traditional CVD risk factors, including serum total cholesterol and systolic blood pressure (Riley et al., 1986). Furthermore, in asymptomatic adult males with metabolic syndrome, those with the worst 10 y CVD risk profile had impaired arterial elasticity compared to those with lower risk profiles (Pohjantahti-Maaroos et al., 2012).

Differences in arterial elasticity can also be seen in childhood where children with hypercholesterolemia display poorer arterial elasticity (measured via Young's elastic modulus) compared to healthy controls (Riggio et al., 2010). Furthermore, brachial artery distensibility declines with the development of obesity and additional impairment is observed in adolescence with both obesity and hyperinsulinemia (Urbina et al., 2007).

2.2.4 Vascular structure

It is well established that an increased cIMT in adults is associated with coronary artery disease, and is predictive of future CVD events (Salonen and Salonen, 1991, Hodis et al., 1998, O'Leary et al., 1999, Dijk et al., 2006). Additionally, traditional CVD risk factors such as age, sex, diabetes mellitus, total cholesterol and smoking are associated with cIMT (Riley et al., 1986, Urbina et al., 2002, Selvin et al., 2005). In adults, cIMT is a valid and reliable measure of CVD risk (Grobbee and Bots, 1994, Greenland et al., 2000).

Early ultrasound studies found children with hypercholesterolaemia had structural changes in their artery with a significantly thicker cIMT compared to healthy control (serum cholesterol <6.4 $mmol \cdot L^{-1}$) children (Pauciullo et al., 1994). Data

from autopsies have shown that atherosclerotic lesions first occur in the abdominal aorta (McGill et al., 2000b). Furthermore, the CVD risk profile in adolescence is predictive of adult cIMT (Raitakari et al., 2003, Laitinen et al., 2012). In contrast, the association between CVD risk in childhood (3-9 y) and adult cIMT is significant but weak in men, and non-significant in women (Raitakari et al., 2003). These data suggest that exposures in youth are potentially more influential for the vascular structural changes than exposures in adulthood, and the legacy of youth exposures cannot be outrun by the adult lifestyle. Furthermore, some of these associations are bi-directional such as the associations between adiposity and carotid-femoral PWV (Agbaje et al., 2022).

Despite the well documented age and sex differences in cIMT in adult populations, only age differences in children and adolescents have been reported (Ishizu et al., 2004, Jourdan et al., 2005). These studies have found that cIMT increases with age.

2.2.5 How do the vascular measures relate to each other?

Although endothelial function and arterial stiffness are both indicators of vascular disease, they are not interchangeable as they measure different aspects of vascular health. Nevertheless, although the two pathological processes are different, some crosstalk does exist. For example, nitric oxide also contributes to arterial compliance (Kinlay et al., 2001). There is a relationship between brachial artery FMD and several measures of arterial stiffness such as proximal aortic compliance (r=0.390; *P*=0.049) in adults with coronary artery disease and individuals presenting with only risk factors but no overt disease (Nigam et al., 2003). A strong inverse correlation between FMD and carotid-femoral PWV

(adjusted for blood pressure) has also been reported in well controlled hypertensive patients (r=-0.74) and normotensive controls (r=-0.83) (Figueiredo et al., 2012). However, a recent study suggests the relationship between FMD, and arterial stiffness may vary depending on the location of the measure (Badhwar et al., 2018). The authors found a significant inverse association between brachial artery FMD and carotid-radial PWV (r=-0.61; *P*=0.0001), but not with carotid-femoral PWV (r=0.9; *P*>0.05) in 37 adults with metabolic syndrome. This suggests that either changes in the vessel are impacting function, or changes in function impact localised structure. The direction needs confirming in future research.

Brachial artery FMD is also inversely associated with cIMT (β=-0.006; *P*=0.001) in adults (Juonala et al., 2004, Halcox et al., 2009). This relationship is stronger than the relationship between traditional risk factors and cIMT in adults free from CVD (Halcox et al., 2009). Furthermore, a significant positive correlation was reported between cIMT and brachial-ankle PWV in a group of hypertensive and normotensive adolescents (r=0.396; P<0.05) (Gil et al., 2008). Similarly, in older adults, (≥60 y old; *n* = 320) a significant positive association (β=0.028; 95% CI = 0001-0.056; *P*=0.045) was found between aortic PWV and cIMT (Del Brutto et al., 2020).

2.3 PHYSICAL ACTIVITY AND SEDENTARY TIME

2.3.1 Definitions of physical activity and sedentary time

Although often used interchangeably in the literature, both PA and exercise, and SB and ST are separate constructs that are not synonymous with each other. Therefore, it is vital that they are defined in PA and ST research. As stated

previously in *Chapter One*, PA is defined as any movement produced by skeletal muscles that results in energy expenditure (Caspersen et al., 1985). In contrast, exercise is a specific type of PA that is planned, structured and repetitive, and has a final or an intermediate objective such as the improvement or maintenance of physical fitness (Caspersen et al., 1985), and therefore it contributes to only a small proportion of total PA. When considering PA, there are a number of dimensions which could be assessed including the frequency, intensity, duration, volume, type and domain (e.g. leisure or work/education) (Strath et al., 2013). PA intensity can be defined in either absolute terms such as in METS, or relative terms such as a percentage of maximum heart rate (Strath et al., 2013). Although PA intensity occurs on a spectrum, often it is separated into three distinct categories: LPA (1.6-2.9 METs), moderate PA (MPA; 3.0-5.9 METs) and vigorous PA (VPA; ≥ 6.0 METs). However, MPA and VPA are regularly combined into one category as MVPA (≥ 3.0 METs).

The term sedentary behaviour describes activities with an energy expenditure ≤1.5 metabolic equivalents involving either sitting, lying or reclining, and ST is the amount of time spent performing SBs (Tremblay et al., 2017) which can include watching television, reading, travel and working. Many of the dimensions of PA such as frequency, duration and type can also be used to describe SB.

Together ST and PA time add to total the time spent awake which is a finite value (Figure 2.2), and this means they are compositional in nature (Dumuid et al., 2018). Physical activity intensity is continuous but when PA is classified into broad, absolute intensity categories (e.g. ST, LPA and MVPA) it is comprised of mutually exclusive parts (Aitchison, 1982b). Typically, MVPA accounts for a very

small proportion of the waking day, with the remainder of time split between ST and LPA (Figure 2.3). Consequently, ST and LPA are highly correlated and there is an inverse relationship between ST and LPA (van der Ploeg and Hillsdon, 2017). A change in the time spent in one of the intensity categories (e.g. MVPA) leads to a change in time spent in one or more of the other components of the composition (e.g. only ST, or both ST and LPA) (Pedisic et al., 2017) so all aspects of the composition should be reported in statistical models.

Wear time between participants is rarely exactly the same, even if a 24-h recording period is used and therefore, adjustments for wear time in analytical models are essential to avoid bias caused by systematic differences in wear time and the incorrect assumption that unmeasured time is allocated equally across the activity behaviours. Collinearity issues occur when all parts of the activity composition and wear time are included in an analytical model because the sum of the activity behaviours adds up to 100% of wear time (Pedisic, 2014). As a result, LPA is often left out of non-compositional multiple linear regression models for PA as it is often the behaviour of least interest for most research questions. The absence of one part of the activity composition means that there are no collinearity issues because the sum of the activity behaviours included do not total to 100% of wear time which is included as a covariate.

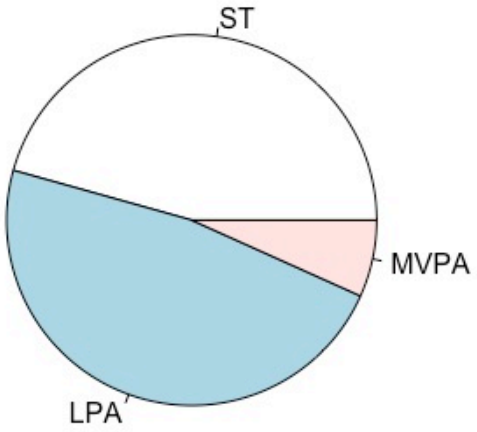

Figure 2.2 – An example composition of the proportion of time spent in each activity behaviour during the waking hours. ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity.

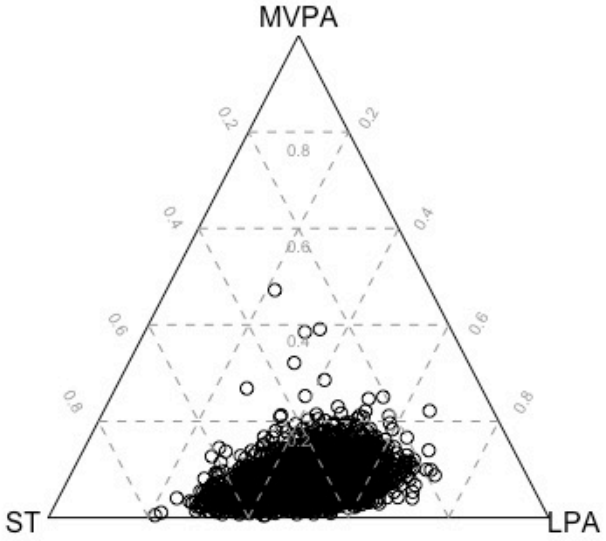

Figure 2.3 – An example ternary plot demonstrating the activity profile of the participants in this thesis with different activity compositions. The closer the point is to the corner, the greater the percentage of the waking day is spent in that activity behaviour. The further away from the corner, the lower the percentage of the waking day is spent in that activity behaviour. In this example, most participants spend the majority of the waking day engaged in ST and LPA, with MVPA contributing for a very small proportion. ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity.

2.3.2 Measurement of physical activity and sedentary time

The measurement of PA and ST is challenging due to the complexity of the behaviours. An accurate measurement method is vital to ensure the reliability and validity of the research. Inaccurate measurement results in underestimating or masking the association between the exposure (PA or ST) and the outcome (e.g. CVD risk factors). Researchers can estimate either PA energy expenditure or metrics of PA, such as total PA time or time in different PA intensities. The measurement method used depends on the research question and the resources available.

Criterion measures of total, and PA energy expenditure include doubly labelled water and indirect calorimetry which estimate energy expenditure from oxygen consumption and carbon dioxide production. These methods are impractical and expensive so are not regularly used to measure habitual PA in research. However, the metabolic chambers for calorimetry can be used to validate the equations for energy expenditure from accelerometers and heart rate, even in paediatric groups (Butte et al., 2000, Janssen et al., 2012).

Direct observation is sometimes considered the gold standard measure for PA and ST measurement in children (Sirard and Pate, 2001). Trained observers will watch participants and record PA and ST at set time intervals, and this method can collect information about all dimensions of PA including intensity and patterning (Hardy et al., 2013). The method is only suited for use in controlled environments for defined periods such as during a school break time or lesson, rather than free-living PA because of the intense nature of data collection for the researcher. There are concerns with the ability of this method to accurately

classify the intensity of PA due to the subjective nature of the method and also the ability to capture the short duration of PA that children engage in if the recording was only taken every minute. Furthermore, it is also open to bias from the Hawthorne effect where the participants may change their behaviour because they are aware of being observed. This method is also not feasible for use in large sample sizes.

Alternative measurement methods of PA and ST can include self-report via questionnaire or interview, and the use of devices such as pedometers, heart rate monitors, accelerometers and inclinometers. The relative strengths and limitations of these methods will be discussed in the following sub-sections.

2.3.2.1 Self-report

A widely used method to assess PA and ST is self-report (Warren et al., 2010) using questionnaires, diaries or interviews. These are often retrospective, so the validity is influenced by an individual's recall ability. Questionnaires are the cheapest and simplest method of assessing PA and ST in a large group of people and allow for the estimation of all dimensions of PA and ST. Questionnaires vary in length and can provide a different level of detail into the activity levels of an individual. The shortest, and least detailed method is a global questionnaire. It broadly categorises an individual (e.g. inactive vs. active) rather than providing precise quantification of PA levels (Milton et al., 2013). Recall questionnaires provide a greater level of detail by quantifying PA and can be used to stratify individuals into more specific PA categories that can relate to PA intensity or PA domain (Strath et al., 2013). Quantitative questionnaires are the most detailed

and can quantify several dimensions of PA and SB and are often conducted via an interview.

Questionnaires can provide qualitative richness to the data which you cannot ascertain from devices, for example what type of PA/SB respondents are doing. Additionally, they can assess some parts of MVPA and broadly categorise the PA level of an individual (Chinapaw et al., 2010). However, questionnaires may mask the true relationships between PA and health outcomes due to their inability to accurately quantify the total volume of PA and PA energy expenditure (Celis-Morales et al., 2012).

There are issues with using questionnaires in paediatric populations. For example, the criterion validity of questionnaires is lower in children (r =-0.25 to 0.41) than for adolescents (r =-0.22 to 0.78) (Chinapaw et al., 2010, Lubans et al., 2011). Very young children (<7 y) are unable to comprehend the questions so cannot complete the questionnaire themselves. Researchers have to rely on parents or teachers to complete the questionnaire which is challenging because adults do not observe all the PA and ST a child engages in, especially when they are observing more than one child, (Bringolf-Isler et al., 2012) so likely underestimate the true values.

Questionnaires can be influenced by bias and this may be intentional (social desirability bias) or unintentional where individuals have errors in the recall of details of their activities such as the intensity or duration (Sallis and Saelens, 2000). This can lead to misclassification of PA or ST and introduces error into the estimates of energy expenditure for an individual. For example, an individual may

underestimate the intensity at which they were walking and report it as LPA rather than MVPA, so their energy expenditure is underestimated. Purposeful exercise is better accounted for than habitual PA since many questionnaires do not ask about LPA or the spontaneous, short duration activities (Tudor-Locke and Myers, 2001). This is especially problematic in children where MVPA is typically short in duration (Baquet et al., 2007) and therefore, questionnaires likely underestimate a child's PA. However, questionnaires can also result in an overestimation of PA compared to device-derived measurements (Basterfield et al., 2008). For example, a mean bias of 122 $min \cdot day^{-1}$ was reported between parental-reported MVPA via questionnaire compared to device-derived MVPA. Overestimating PA could lead to the inflation of the effect of an intervention and may result in an intervention being implemented into policy that is not as effective at increasing PA as it appears.

2.3.2.2 Accelerometers

With advances in technology, many researchers have switched to using devices such as accelerometers which measure acceleration, and this is used to estimate PA and ST to avoid some of the issues associated with the self-report methods. Accelerometers are small devices that detect acceleration (change in velocity) of the body, or body segment to which they are attached. The intensity of the movement of a body part can be calculated from the acceleration data, along with the frequency and duration of movement (Chen and Bassett, 2005, Corder et al., 2008). Depending on the device, they can measure acceleration in one (uniaxial), two (biaxial) or three (triaxial) directions.

Accelerometers measure movement acceleration and until the last 10 y or so devices from different manufacturers provided arbitrary proprietary summaries in the form of accelerometer data counts or counts per minute (counts·min^{-1}). Counts are arbitrary values that are not comparable between devices. Furthermore, counts are influenced by the filtering procedures applied to the data as well as the amplitude and frequency of acceleration (Matthews, 2005, Reilly et al., 2008). Therefore, the use of counts limits the comparability of results between different devices and studies. Newer devices provide the raw accelerometer data in units of gravity, and these data can be summarised using metrics such as Signal Magnitude Area (Khusainov et al., 2013), Euclidean Norm Minus One (van Hees et al., 2014) and Mean Amplitude Deviation (Aittasalo et al., 2015).

Energy expenditure and time spent in each of the intensity categories can be calculated from counts·min^{-1} by applying cut-points. Numerous calibration studies have been performed to derive energy expenditure equations and intensity cut-points from the accelerometer data in counts per minute by measuring oxygen consumption for different activities performed in laboratory conditions (Mattocks et al., 2007, Evenson et al., 2008, Pulsford et al., 2011). This allows the relationship between movement and energy expenditure to be quantified. However, some studies only include a limited number of activities such as sitting, walking and jogging so the equations and intensity cut-points may not be representative of typical activities performed across the PA intensity spectrum. Additionally, not all activities conducted in the laboratory accurately reflect activities performed in free-living conditions such as treadmill activities performed on no gradient underestimate free-living PA energy expenditure. The cut-points

are therefore specific to the sample on which they were calibrated and therefore, may not be appropriate for use in individuals who are of a different age or fitness status. Time spent in each of the intensity cut-point ranges are summed to determine the amount of time spent in each PA intensity per day. The calibration of cut-points is also specific to the epoch length of the data from which they were derived. Therefore, applying cut-points that have been validated on a different epoch length (e.g. 60 s epoch) to the data (e.g. 30 s epoch) results in either an under or overestimation of time spent in each of the activity behaviours (Banda et al., 2016). At present, there is no consensus on which cut-points to use for PA thresholds or for ST which means the definitions of these activity intensities varies between studies and makes comparisons of findings challenging because the use of different cut-points can produce vastly different results in the number of minutes per day spent in each activity intensity (Trost et al., 2011).

The counts from an accelerometer are typically summed over a set period such as 10 s or one min, and this is known as an epoch. The choice of epoch used influences the results. For example, a one min epoch has been shown to result in an underestimation of MPA, VPA and MVPA in adolescents (Sanders et al., 2014). Specifically, leisure time VPA was 11.6 ± 11.2 min·day^{-1} using a 1 s epoch but 3.4 ± 6.2 min·day^{-1} using a one min epoch. Children and adolescents typically engage in physical activity that is sporadic, short duration and of a variable intensity in nature (Hoos et al., 2004). Summarising sporadic PA over long periods (e.g. 60 s epochs) lowers the average acceleration value for the measurement period because the peaks in acceleration from the short duration PA are brief. Therefore, to increase validity of the PA estimates, it has been suggested that shorter epochs are preferable, with an epoch between 1 and 10 s

for children (Rowlands, 2007). Shorter epochs were not available in older devices. MPA and VPA are sometimes combined into one category as MVPA because of the underestimation of MPA and VPA with 1 min epochs compared to shorter epochs (Sanders et al., 2014). However, a 1 min epoch has recently been shown to result in better estimate of ST in children and adolescents compared to shorter durations such as 5 or 15 s epochs (Altenburg et al., 2021). Therefore, the outcome of interest should be taken into account when deciding on the most appropriate epoch to select.

Devices can be worn on the hip, wrist, thigh or ankle and the location of the device influences the estimates of a given output, even for the same activity (Ekblom et al., 2012, Routen et al., 2012, Hildebrand et al., 2014). The placement of the device has also been shown to influence the compliance of individuals wearing the devices. For example, in a group of 9-10 y old children, compliance was higher when the device was worn on the wrist compared to when it was worn on the hip (Fairclough et al., 2016). A total of 97 children out of 109 meet wear time inclusion criteria of 3 d (including 1 weekend day) with a minimum of 10 h of wear time using the wrist worn device but only 84 met this criteria with the hip-worn device. Therefore, studies which have used a hip-worn accelerometer may be affected by increased data loss compared to those that use a wrist-worn accelerometer.

The hip-worn devices are also unable to determine posture, and because the definition of SB is dependent on posture (Sedentary Behaviour Research, 2012), this results in misclassification of standing and even some LPA as ST, meaning ST is likely to be overestimated. Additionally, some devices are not waterproof

meaning they are unsuitable for quantifying water-based activities such as swimming. They are also not suitable for contact sports and can have issues detecting cycling. Consequently, this can result in an underestimation of MVPA in some individuals engaging in these types of activities.

As an intensity category, MVPA is very broad and includes all activity ≥ 3 METs. There is no upper intensity limit on the traditional MVPA category so an individual can go from a brisk walk to maximal intensity sprinting without changing the time spent in MVPA. The influence or associations between time spent in the different intensities within the broad MVPA category on an outcome measure (e.g. vascular outcomes or CVD risk factors) could be different so by combining these together, associations could be masked. For example, a dose-dependent inverse association has been reported between the magnitude of the associations reported between time spent at increasingly high intensities within the MVPA category and skinfold thickness (Collings et al., 2017). Additionally, most accelerometers provide no detail on the type of activity an individual is engaging in e.g. running vs. cycling, although activPAL devices when worn on the thigh are able to detect time spent cycling.

The standard approach of reporting PA or ST in minutes per day also gives no detail on the richness of the data in terms of patterns in how PA and ST is accumulated throughout the day (e.g. workplace vs. leisure), which may be an important factor for health outcomes (Gupta et al., 2020a). However, researchers can quantify the patterning of PA and ST using these devices and the literature is beginning to examine the influence of PA and ST patterning on health outcomes.

Although the use of devices to measure PA and ST is less affected by the biases associated with self-report, individuals may still change their behaviour because they are wearing them (Hawthorne effect) by engaging in more PA than normal which can influence the validity of the results. In children and adolescents, the first day of recording is normally the most active and the start day of recording has also been shown to affect the estimate of PA (Dössegger et al., 2014). This has led to the suggestion that researchers should consider including a familiarisation period to wearing the devices (one day in school-age children and two in pre-schoolers) and discard this data, and to also randomise the start day to increase validity of the results (Dössegger et al., 2014).

2.3.2.3 Inclinometers

Inclinometers can also measure PA and ST. These are small, lightweight, devices typically attached to the midline of the quadriceps. Time spent in different postures such as sitting and standing is derived by assessing the thigh orientation with respect to gravity. Inclinometers can provide a valid quantification of time spent in ST as well as PA in all age groups, including children (Davies et al., 2012, Dowd et al., 2012). There has been some misclassification of time spent sitting as standing using inclinometers (Davies et al., 2012), meaning ST can be underestimated. However, measurement of SB using devices on the thigh is more appropriate than assessing SB with a waist-worn device because the waist-worn devices cannot determine the difference between sitting and standing at all. Inclinometers are expensive so may not be feasible for use in studies with large sample sizes. Additionally, inclinometers cannot provide any contextual information for what activity the individual is engaging in during that posture, for

example whether they are reading a book or watching television during a sitting bout. Future research should combine self-report activity diaries with devices to add the qualitative contextual detail that devices are unable to capture.

2.3.3 Physical activity in childhood and adolescence

The most recent data from the Active Lives Survey (parental or child questionnaire responses) reported that 44.9% of 5-16 y olds in England met the PA guidelines of an average of ≥60 min of MVPA per day (Sport England, 2021). However, this data was affected by the Coronavirus-19 pandemic where schools closed for most children and adolescents, leisure centres were shut, and sports clubs were cancelled. Therefore, although this data represents PA levels at the time, this likely does not reflect habitual PA levels of children and adolescents because government restrictions at the time were limiting opportunities for children and adolescents to engage in PA. Consequently, the results of the 2018-2019 survey will be discussed in this thesis as they were not disrupted by the impacts of the Coronavirus-19 pandemic so likely better reflect current habitual PA levels of children and adolescents in England.

Data from the Active Lives Survey 2018-2019 reported that 47% of 5-16 y olds met the PA guidelines (an average of ≥60 min of MVPA per day) (Sport England, 2019a). Boys were more likely to be meeting or exceeding the guidelines than girls (51 vs 43%, respectively). Although collected at an earlier date, device-derived data also shows sex-differences in PA which is in line with the results from the Active Lives Survey. For example, sex differences have been reported in the time spent in the PA intensities in children aged 9-10 y (Steele et al., 2009), with boys reported to be more active than girls (84.2 ± 25.9 vs. 66.1 ± 20.8

min·day^{-1} MVPA, respectively). Furthermore, a greater percentage of boys (81.5%) aged 9-10 y were reported to meet the PA guidelines of ≥60 min of MVPA per day compared to girls (59.4%) (Steele et al., 2009).

The results from the 2018-2019 Active Lives Survey reported that secondary school (Years 7-11) pupils were more likely to be meeting the PA guidelines than primary school (Years 3-6) pupils (46.3% vs. 44.3% of boys and 38.5% vs. 37.8% for girls, respectively) (Sport England, 2019b). This is at odds with other published data which demonstrates that during the adolescent years, physical activity declines (Dumith et al., 2011). This decline is observed in both boys and girls, and the decline in total PA begins to occur by age 7 y in UK children (Farooq et al., 2018). However, it is important to highlight that this decline in PA was not observed for all, with 19% of boys who had relatively high MVPA remaining stable from 7 to 17 y. Furthermore, an age by sex interaction exists in the decline in total PA from 9-15 y (Metcalf et al., 2015). The difference in data between the Active Lives Survey and other published data might be because of differences in how the data was collected. Questionnaire methods for assessing PA in children and adolescents are known to be subject to reporting bias (Basterfield et al., 2008) and the participants were different at the different age groups. In contrast, studies reporting the decline in PA levels have measured the same participants over a number of years (Dumith et al., 2011, Metcalf et al., 2015, Farooq et al., 2018) and some have used device-derived measurements which may reflect habitual PA levels more accurately as they are not subject to reporting bias.

2.3.4 Sedentary behaviour and time in childhood and adolescence

In UK children and adolescents, device-derived ST (≤25 counts/15 s via Actigraph accelerometers) increases from age 7 to 15 y (346.5 ± 66.6 to 535.4 ± 85.4 min·day^{-1}, respectively) (Janssen et al., 2016). However, due to the nature of this device, there is likely some error in the exact values due to the inability of the device to determine posture and therefore it will likely include some time spent standing or in very slow LPA. Additionally, the average duration of the bout length (defined as the minimum period of ST without allowing any interruption i.e., no counts >25 count·15 s^{-1}) increased over the course of the measurement period from 3.4 ± 3.8 min to 7.3 ± 3.9 min. This shows that adolescents are not only more sedentary on average than children but they also accumulate this ST differently from bouts of a longer duration. Recent child and adolescent data (*n*=78; 6-17 y old) from activPAL devices reported that all the children and adolescents sat uninterrupted for periods of up to 30 min and engaged in at least one bout of uninterrupted sitting between 30 min to 1 h in duration. There are also sex differences present in accelerometer determined ST (time spent below 100 CPM), with UK girls aged 10 engaging in more ST per day than boys (450 ± 56.2 vs. 461 ± 51.6 min·day^{-1} ST, respectively) (Steele et al., 2009). Questionnaire-measured ST (excluding school hours) was reported to be higher on weekdays in 11-15 y olds (3.9 h; *n*=815) compared to both 5-10 y olds (2.7 h; *n*=1065) and 2-4 y olds (2.7 h; *n*=555) in the UK in 2015 (Scholes, 2016). In addition, ST is more prevalent at weekends than weekdays for adolescents (4.6 h vs. 3.9 h) but these data do not include ST while at school, which could be biasing the results. During ST, children and adolescence engage in numerous SBs such as watching television, playing computer games, reading, completing schoolwork and passive commuting. Sex differences in total screen time were reported in data from

51,922 Canadian students in grades 6 to 12 with boys spending more time engaged in screen time than girls (8.3 ± 2.5 $h \cdot d^{-1}$ vs. 7.3 ± 2.1 $h \cdot d^{-1}$, respectively) (Leatherdale and Ahmed, 2011). There is also an effect of age on screen time in children and adolescents from North West England, with daily self-reported screen time (watching television, playing video games, using computers or tablets, and using a mobile phone) at 581.09 ± 107.81 $min \cdot day^{-1}$ in Year 5 students (*n*=93), 671.96 ± 112.59 $min \cdot day^{-1}$ in Year 8 (*n*=94) students and 725.80 ± 115.20 $min \cdot day^{-1}$ in Year 10 (*n*=105) students (Noonan et al., 2019). These values appear high but may be affected by the questions which did not specify whether these devices were being used simultaneously for some of the time. In addition, there are sex differences in the time spent in different types of SBs reported by UK children (*n*=1,513) with 10 y old girls spending more time in non-screen SBs (e.g. reading, doing homework or arts and crafts) than boys, and boys spending more time in screen-based SBs (e.g. playing videogames and watching television or videos) than girls (Klitsie et al., 2013).

A recent systematic review and meta-analysis reported device measured ST increased with age, but there was no evidence of the magnitude of change varying by age or sex (Kontostoli et al., 2021). However, only a limited number of studies had the required data for the analysis. In addition, it was reported that screen-based ST (television viewing, video game playing and computer use) measured through questionnaires increases with age, with a weighted mean difference of 20.8, 19.9, 40.0 and 42.6 $min \cdot day^{-1}$ over a 1, 2, 3 and 4+ y follow up. Data from a recent review also suggests some of the time that was spent watching television in early research may be being replaced by spending time on newer devices such as tablets and smartphones (Thomas et al., 2020).

Consequently, the risk estimates from older data where time spent on the newer technologies was minimal, may not accurately reflect the risk of these behaviours in modern times so contemporary data are required.

2.4 TRACKING OF ACTIVITY BEHAVIOURS

2.4.1 Tracking of physical activity

Small to moderate tracking coefficients (range 0.27 to 0.57; median 0.36) for physical activity outcomes (counts per min, ≥2500 counts·min^{-1}, direct observation, number of PA min that PA heart rate ≥50% of individual resting heart rate and direct observation) have between reported between early (0-5.9 y) to mid- (6-12 y) childhood (Jones et al., 2013). In UK children, the tracking coefficients for total PA and MVPA between 5-15 y was 0.27 and 0.16 respectively (Metcalf et al., 2015). Tracking of multiple domains of PA (e.g., leisure or transport PA) from childhood to adulthood is weak (-0.08-0.14) (Cleland et al., 2012). Although these data suggest that early intervention is essential, due to the weak nature of the tracking coefficient from childhood to adulthood, some people will become more, or less active. Therefore, identifying the factors that influence why an individual changes their behaviour is necessary for developing effective interventions.

2.4.2 Tracking of sedentary behaviour

Moderate to large tracking coefficients (range 0.35-0.60 and median 0.52) of SBs have been reported during early childhood and from early (0-5.9 y) to mid- (6-12 y) childhood (Jones et al., 2013). Data from 5,991 children and adolescents (aged 4-17 y) from the ICAD reported moderate tracking coefficients for both total (0.48) and uninterrupted sedentary time (0.43) (van Ekris et al., 2020). Similarly,

moderate tracking coefficients exist for individual SBs such as television viewing and playing computer games during childhood and adolescence, and into adulthood (Biddle et al., 2010). Therefore, interventions for the primary prevention of CVD need to be targeted at the age of childhood before the habit of undertaking large volumes of ST, engaged in specific SBs that are associated with increased risk or poorer health outcomes such as television viewing (Ekelund et al., 2006, Carson and Janssen, 2011, Chaput et al., 2013, Rey-Lopez et al., 2013, Wennberg et al., 2013, Grontved et al., 2014, Vaisto et al., 2014, Barker et al., 2018, Noonan et al., 2019, Vanderloo et al., 2020) becomes the norm and persist into adulthood. However, it is important to note the tracking of some SBs may not be such an issue for health later in life. For example, high computer usage in childhood and adolescence (if used for studying) may not be a problem if this leads to a well-paid office job as an adult. High total sitting time in this case might not be as big of a problem as first thought because of the decreased risk of diseases associated with higher socioeconomic status (Kivimaki et al., 2020). In contrast, engaging in high television or video game time in both childhood and adulthood is likely to be detrimental for health from the co-occurring behaviours such as increased caloric consumption (Pearson and Biddle, 2011, Thivel et al., 2013, Shang et al., 2015). Therefore, researchers may need to consider what specific activity individuals are doing on screens, rather than labelling all screen time as harmful.

2.5 PHYSICAL ACTIVITY, SEDENTARY TIME AND CARDIOMETABOLIC RISK IN PAEDIATRIC POPULATIONS

2.5.1 Physical activity

Analysis of data from 67 children (10-12 y old) revealed that inactive children (<60 min MVPA per day) had higher clustered CMR score, higher ST, and lower cardiorespiratory fitness in comparison to the active children (≥60 min MVPA per day) (Boddy et al., 2014), which is unfavourable for health. Furthermore, total PA (questionnaire measured) was inversely associated with a clustered CMR score and individual CMR factors: body fat percentage, waist circumference, fasting insulin, glucose, TAG, HDL cholesterol and TAG, very-LDL cholesterol and TAG in 468 children aged 6-8 y old, (Vaisto et al., 2014). Eight of these associations are favourable for health and one (HDL cholesterol) is unfavourable. Similarly, low levels of accelerometer determined total PA from 2,049 primary school children in Year 5 (9-10 y old) from 78 schools was unfavourably associated with increased CMR, ponderal index (a marker of adiposity), waist circumference, sum of skinfolds and fat mass index (Owen et al., 2010). Additionally, total PA (accelerometer measured) was inversely associated with SBP, DBP, insulin, glucose, TAG, HDL, and a clustered CMR score in 1,092 children aged 9 y old, and 829 adolescents aged 15 y old (Ekelund et al., 2006). Six of these associations are favourable for health and one (HDL) is unfavourable. A graded negative (favourable) association was also reported in 1,725 girls and 1,592 boys aged 9 and 15 y old between the clustering of risk factors with PA (mean counts·min^{-1}) (Andersen et al., 2006). Furthermore, total PA (total accelerometer counts) was inversely (favourably) associated with metabolic risk in 589 children with an average age of 10 y old (Brage et al., 2004). Analysis of longitudinal data from 113 boys and 99 girls from Plymouth, UK who were measured annually

between 5 y and 8 y found the average number of minutes ≥3 METs (across the four time points) was not significantly associated with changes in measurements of body mass or body fatness in either boys or girls (Metcalf et al., 2008). There was a significant inverse partial correlation (r=-0.23, *P*=0.03) between the average number of minutes ≥ 3 METs and the change in the composite metabolic z-score in the girls, but the partial correlation was not statistically significant in the boys (r=-0.17, *P*=0.07).

No significant correlation was found between LPA, MVPA or VPA with a CMR score in 100 children and adolescents (10-14 y old) (Bailey et al., 2012). However, the authors found that LPA was positively correlated with WC and body fat percentage, whereas VPA was inversely correlated with DBP and body fat percentage. The LPA associations are unfavourable for health, but the VPA associations are favourable for health.

In contrast, MVPA was inversely associated with CMR factors in a large sample of children and adolescents (*n*=20,871; aged 4-18 y) (Ekelund et al., 2012). In adolescent boys (*n*=376; 13-18 y old) MVPA (accelerometer determined) was also inversely associated with a clustered CMR score (Rey-Lopez et al., 2013). When MPA and VPA are examined separately, accelerometer determined LPA, MPA and VPA (% of wear time) were all inversely associated with SBP, DBP, insulin concentration and the CMR risk score in data from 1,709 9-10 y olds and 15-16 y olds (Ekelund et al., 2007). Additionally, MPA and VPA were inversely associated with glucose concentration and MPA was also inversely associated with TAG concentration. In 605, 9-17 y old children and adolescents VPA but not MPA was significantly inversely associated with BMI z-score, SBP and positively

associated with maximal oxygen uptake (Hay et al., 2012). Furthermore, VPA (but not MVPA) was inversely associated with a CMR z-score in adolescents (Ried-Larsen et al., 2014). The associations reported for MPA, VPA and MVPA are favourable for health.

Recent analysis of cross-sectional accelerometer data from 29,734 children and adolescents (4-18 y old) revealed it was time spent in higher intensity PA rather than bout-duration that was the main reason for variation in CMR (Tarp et al., 2018). The magnitude of the inverse association between PA and CMR risk was greater with the higher intensity PA, suggesting promoting PA of higher intensity in children and adolescents should be the priority.

From the prospective analysis of data from 841 children and adolescents aged 9-15 y at baseline with follow up yearly for 2 y, an inverse dose-response relationship was observed between MPA and waist circumference (Carson et al., 2013). For VPA, there was a dose-response relationship with a higher maximal oxygen uptake at follow up. The associations between baseline VPA and follow up waist circumference and SBP were also examined with the data stratified by sex. This revealed a sex difference in the association, with a dose response relationship between baseline VPA and a decrease in follow up waist circumference and SBP in boys but not in girls. A dose-response relationship was also observed between LPA at baseline with an increase in the conditional BMI z-score velocity, BMI z-score and waist circumference at follow up. Furthermore, VPA but not MVPA in childhood (age 9 y, *n*=251) was inversely associated with CMR in adolescence 6 y later (Ried-Larsen et al., 2014). A recent meta-analysis

of prospective data has also found a small but significant inverse association between MVPA and clustered CMR in youth (Skrede et al., 2019).

2.5.2 Sedentary behaviour and time

A significant positive (unfavourable) association was reported between ST (% of wear time) with a clustered CMR score and individual CMR factors (SBP, DBP, TAG, glucose and insulin concentration) in 1,709 9-10y old and 15-16 y old children and adolescents (Ekelund et al., 2007). However, when time spent in MVPA is controlled for, ST was not associated with CMR factors (Ekelund et al., 2012), suggesting the association between ST and CMR only existed due to residual confounding variance from differences in time spent in MVPA between participants. The absence of a statistically significant association between ST and CMR factors has been reported in other studies (Hay et al., 2012, Barker et al., 2018). Additionally, the volume and pattern of ST (bouts of SB ≥ 30 min and breaks in bouts of SB ≥ 30 min) was not associated with increased CMR after adjustment for device determined MVPA in 2,527 children and adolescents aged 6-19 y old (Carson and Janssen, 2011). A contemporary systematic review has also reported no prospective associations between device-derived ST and CMR factors (Skrede et al., 2019). Research has adjusted for a variety of confounding variables in the analytical models with some including wear time, but none have included LPA. The unadjusted variance from LPA is likely introducing error into these reported associations.

Subsequent analyses have shown that it is certain SBs (e.g., television viewing) rather than total ST that are positively associated with CVD risk when other factors such as diet, other PA intensities and socioeconomic status are controlled

for (Ekelund et al., 2006, Carson and Janssen, 2011, Chaput et al., 2013, Rey-Lopez et al., 2013, Grontved et al., 2014, Vaisto et al., 2014, Barker et al., 2018, Noonan et al., 2019, Vanderloo et al., 2020). A recent systematic review reported screen time and television viewing were consistently positively associated with CMR scores which is unfavourable for health, but there are inconsistent or null findings for most other SBs (Carson et al., 2016a).

Not all screen-time SBs are associated with the same risk either, with high television viewing but not computer usage associated with increased CMR when MVPA and other confounders such as age, sex, ethnicity, SES, smoking status, total fat, saturated fat, cholesterol and sodium intake are controlled for (Carson and Janssen, 2011). The study reported that children and adolescents who watched television for ≥4 h per day were 2.53 times more likely to have increased CMR than those who watched <1 h per day. The difference in associations between television viewing and computer usage may be in part due to residual confounding variance from socioeconomic status. Children from poorer families are more likely to spend more than 1 $h \cdot d^{-1}$ watching television than their wealthier peers, and they are also less likely to be participating in sports (Fairclough et al., 2009). Therefore, poorer children may spend more time watching television because of a lack of access to sports clubs due to cost so there is a double negative of engaging in less MVPA and spending more time sedentary. The difference in risk between the SBs could also be due to co-occurring behaviours such as increased caloric consumption from snacking (Pearson and Biddle, 2011, Thivel et al., 2013, Shang et al., 2015) via mechanisms such as “mindless eating” (Boulos et al., 2012) and from the consumption of energy dense foods e.g. salty snacks and biscuits (Pearson et al., 2014). Contemporary data are required that

reflect the change in device use over recent decades such as the increase in use of portable screens including the use of smartphones and tablets to watch films and episodes on-demand rather than traditional television screens (Saunders and Vallance, 2017). Adolescents also often use multiple devices simultaneously (Harrington et al., 2021) so measurement tools need updating to account for this.

Some SBs such as homework have been found to have a favourable relationship with health outcomes, for example, an inverse association was reported between the duration of homework and SBP in 2,353 12 y old students (Gopinath et al., 2012). Furthermore, musical instrument use was inversely associated with fasting insulin and listening to music positively associated with HDL cholesterol concentration in 468 children (6-8 y old) (Vaisto et al., 2014). Therefore, this suggests that not all SBs have negative health consequences, but this could simply be due to differences in the pattern of the behaviour that is ignored by an average time based metric. For example, it may be that children take more standing or PA breaks when doing homework compared to watching television which is altering the associations with the health outcomes between these difference SBs. Additionally, the differences in associations between SBs are likely largely due to residual confounding from differences in the co-occurring behaviours or from socioeconomic factors since the sedentary stimulus for cardiometabolic markers should be the same in all SBs if there are no differences in the pattern in which the time is accumulated.

Nevertheless, SB is an emerging risk factor for cardiometabolic disease in children (Saunders et al., 2014) and has been unfavourably associated with individual CVD risk factors such as obesity, hypertension, insulin resistance,

elevated blood glucose and lipid concentrations in cross-sectional studies (Sardinha et al., 2008, Vaisto et al., 2014). Therefore, the associations between ST and individual SBs with CVD risk factors require further investigation and should include additional metrics of ST such as the number of breaks in ST and duration of sedentary bouts to provide a richer understanding of the many facts of SB (e.g. intensity, duration and frequency). The analysis of prospective data should also be included to strengthen the evidence base.

2.5.3 Limitations and methodological considerations of the existing literature

Collectively, data for the associations between PA and ST with CMR provide evidence to support the recently updated PA guidelines (UK Chief Medical Officers', 2019). However, there is a lack of data demonstrating the benefits of LPA in children to support the recommendation of replacing ST with PA of at least a light intensity so more research into the benefits of LPA in childhood is required. This is especially important since MVPA contributes to such a small proportion of the waking day and LPA is likely to be more amenable and accessible to individuals to engage in. Additionally, some of the existing literature fails to account for time spent in all the remaining activity behaviours. For example, Carson et al. (2013) did not control for ST in the analytical models whereas Ekelund et al. (2012) did not control for LPA and therefore, error will exist in the associations from differences in ST or LPA between participants.

The research that adds the time spent in the remaining PA behaviours as covariates also has limitations because there is failure to include wear time in the analytical models (Ekelund et al., 2006, Hay et al., 2012, Barker et al., 2018).

Wear time is a necessary covariate because differences in wear time can artificially drive differences in measured behaviour between participants, especially ST (Tudor-Locke et al., 2011) and likely introduces error into the observed associations.

Using a non-compositional multiple linear regression with time spent in the activity behaviours measured in minutes per day, it is not possible to include time spent in all the activity behaviours and wear time in the analytical model due to collinearity issues because the sum of the activity behaviours is the wear time (Pedisic, 2014). Therefore, to include wear time, one of the activity behaviours must be removed from the analytical model (often LPA), and this leads to error in the associations from not accounting for the influence of that behaviour on the outcome. Alternative analysis methods, such as compositional data analysis (CoDA) (Aitchison, 1982b, Chastin et al., 2015, Dumuid et al., 2018), allows for adjustment of all activity behaviours and the variation in wear time so likely produces an association with less error than the non-compositional multiple linear regression approach.

Although many of the study samples in the literature examining associations between PA and ST with CMR are relatively large (*n*=292-20,871), error is introduced from the use of samples that are heterogenous in age spanning across childhood into late adolescence (4-18 y old) (Andersen et al., 2006, Ekelund et al., 2006, Ekelund et al., 2012, Hay et al., 2012, Carson et al., 2013, Noonan et al., 2019, Vanderloo et al., 2020) or across adolescence (13 to 18 y old) (Rey-Lopez et al., 2013, Barker et al., 2018). With the well-established differences in PA and ST levels throughout childhood and adolescence (Dumith et al., 2011,

Janssen et al., 2016, Farooq et al., 2018), it is important to examine the associations between PA and ST with CMR either at specific ages or within childhood and adolescent groups.

Finally, current evidence is largely limited to the study of traditional CVD risk factors such as hypertension and blood lipids, and there are other novel risk factors to consider such as the vascular outcomes, which may add explanatory power. Therefore, research should consider also examining novel risk factors alongside the traditional ones.

2.6 THE "RISK FACTOR GAP"

The Bogalusa Heart Study found that individuals without risk factor exposure had lesion development which may indicate that the traditional risk factors they assessed do not account for all of the structural changes observed (Berenson et al., 1998). Therefore, other "novel" risk factors might be responsible for the changes exhibited in the individuals without traditional CVD risk factor exposure and the additional risk from the "novel" risk factors requires further investigation (Balagopal et al., 2011).

Improvements in traditional CVD risk factors account for 60% of the associated decrease in CVD risk from high levels of PA in adult women (Mora et al., 2007). This has led to the exploration of changes in "novel" risk factors. For example, changes in endothelial function have been reported after a variety of exercise training programmes (Green et al., 2003, Joyner and Green, 2009, Bond et al., 2015a). In adolescents, a two-week high intensity interval exercise program only reported significant changes in the novel risk factors (endothelial function and

heart rate variability) and not the traditional risk factors (Bond et al., 2015a). This phenomenon has also been observed in adult literature (Green et al., 2003). These data suggest that changes in endothelial function likely precede alterations in other CVD risk factors, making vascular function an important outcome to target with interventions. However, the direction needs confirming as it may be bi-directional as observed for associations between adiposity and carotid-femoral PWV in the ALSPAC sample between 17 and 25 y (Agbaje et al., 2022). Together this highlights the importance of including assessment of changes in "novel" risk factors alongside the traditional CVD risk factors in both observational and interventional research.

2.7 PHYSICAL ACTIVITY, SEDENTARY TIME AND VASCULAR HEALTH

2.7.1 Physical activity and sedentary time exposure in youth and the associations with adult vascular health

2.7.1.1 Physical activity

Research has found favourable associations between self-reported PA (especially VPA) in late adolescence/early adulthood with arterial elasticity and compliance in adulthood (Palve et al., 2014, van de Laar et al., 2011). It has also been reported that lifetime VPA (measured via an interview between age 13 and 36 y old) but not LPA or MPA is favourably associated with carotid stiffness age 36 y old (van de Laar et al., 2010). However, the participants were diverse in age in the study by Palve et al. (2014) with 1,417 children aged 9 to 15 y, and 999 young adults aged 18 to 24 y at baseline included in the analysis. Additional research is required to examine these associations in samples that are more homogenous in nature and use device derived measures of PA to confirm these findings.

2.7.1.2 Sedentary behaviour and time

In a sample of 373 adults aged 36 y, television time measured 4 y previously (via questionnaire) was associated with higher arterial stiffness (van de Laar et al., 2014). On average, the participants with the stiffest arteries spent an additional 20 min·day^{-1} watching television compared to the participants with the least stiff arteries. The association between increased television viewing and a stiffer artery remained after the addition of VPA and other CVD risk factors such as adiposity and blood pressure as covariates. Further analysis showed the significant unfavourable association between increased television viewing and increased arterial stiffness could only be partially explained (30%) by unfavourable associations between television viewing time and other CVD risk factors including cardiorespiratory fitness, adiposity, and blood pressure. This suggests that television viewing has a direct influence on vascular health but the mechanism by which this occurs is not understood at present.

2.7.2 Paediatric evidence

2.7.2.1 Associations between physical activity and vascular structure and function in children and adolescents

A recent systematic review on the associations between MVPA or MPA or VPA and different vascular outcomes in children and adolescents found significant favourable associations in numerous studies (Baumgartner et al., 2020). For example, there have been significant positive associations reported between habitual PA (Abbott et al., 2002), VPA (Hopkins et al., 2009b) and leisure-time PA (Pahkala et al., 2011) with endothelial function. A significant positive association has been reported between LPA, MPA, MVPA but not VPA with

arterial compliance in 8-11 y old children (Nettlefold et al., 2012). Inverse associations between total PA (Chen et al., 2012), unstructured PA (Veijalainen et al., 2016), MPA and VPA (Haapala et al., 2017) with arterial stiffness have been reported. In addition, significant positive correlations have been reported between MVPA and MPA with cIMT in 54 adolescents aged 13-17 y old (Ascenso et al., 2016), and a significant inverse association was reported between leisure-time PA and aIMT in 13-17 y old adolescents (*n*=1,578) (Pahkala et al., 2011). However, a few studies in the systematic review reported no significant associations (Melo et al., 2015, Reed et al., 2005, Ried-Larsen et al., 2014, Ried-Larsen et al., 2013, Heil et al., 2020). The studies reporting favourable associations were more likely to have used a subjective measurement (questionnaire) of PA, which are prone to reporting bias in which MVPA is significantly over-estimated (Basterfield et al., 2008) and may inflate the relationships with the outcomes. Inconsistencies in the results could also be due to the inclusion of different covariates in the analyses. For example, Heil et al. (2020) found VPA was inversely associated with PWV in the unadjusted model, but not after the inclusion of the following covariates: age and sex-specific BMI-standard deviation score, age, sex and wear time. This highlights the necessity to include a comprehensive set of key covariates into the models, but current literature has been restricted in its ability to do this by small sample sizes. Other key covariates such as lean mass and cardiorespiratory fitness (Sletner et al., 2018, Agbaje et al., 2019) have often failed to be included in the current literature, which could be further inflating the observed associations.

Much of the existing literature is cross-sectional in nature so the direction of causality cannot be determined. However, a recent prospective study of children

aged 6-9 y at baseline with a two y follow up period also noted an increase in VPA was associated with a better arterial dilation (in response to an exercise bout), and the two y change in MVPA was inversely associated with arterial stiffness (Korhonen et al., 2021). Additional prospective data are required and in order to tease out the direction of causality of the associations future studies should also perform a cross lagged analysis.

2.7.2.2 Associations between sedentary behaviour and vascular function or structure

A recent systematic review summarised there is an absence of statistically significant associations between ST and the vascular outcomes in children and adolescents (Konigstein et al., 2020). However, it was highlighted that the current evidence for associations between ST and vascular outcomes is predominantly of low quality and limited by small sample sizes and a lack of control of key confounding variables such as wear time or time in the other activity behaviours in the statistical analyses (Konigstein et al., 2020). For example, despite the significant relationships between SBs and CVD risk, a study has shown that brachial artery FMD was not associated with total ST in 10 y old children (Hopkins et al., 2012). Due to the relatively small sample size (116 children), Hopkins et al. (2012) were unable to control for key covariates such as lean mass (Sletner et al., 2018) and cardiorespiratory fitness (Agbaje et al., 2019) which could have impacted the findings of this study from residual confounding variance masking significant associations between ST and FMD. The narrow age range (10 ± 1 y) of the participants also prevents us from understanding if there is a relationship between ST and endothelial function in adolescents, or how this relationship may alter with changes in age and pubertal status. Furthermore, no significant cross-

sectional associations have been reported between ST and measures of vascular stiffness and structure in both children (Nettlefold et al., 2012, Melo et al., 2015, Veijalainen et al., 2016, Haapala et al., 2017) and adolescents (Ascenso et al., 2016). However, in some studies, ST was measured via subjective questionnaires which often have low reliability and validity (Lubans et al., 2011), leading to underestimates of ST compared to device-derived ST from accelerometers (Veijalainen et al., 2016, Fujiwara et al., 2018). The absence of significant associations reported in some studies could also be because the children were highly active (average MVPA >110 min·day^{-1}) and engaged in very little ST so the associations may be population specific (Nettlefold et al., 2012, Haapala et al., 2017). Therefore, these results cannot be generalised to other paediatric populations who are habitually sedentary and not regularly meeting PA guidelines.

A significant unfavourable longitudinal association between increased time spent watching television and a higher brachio-ankle PWV has been reported in 14-15 y old male Japanese students (Fujiwara et al., 2018). This suggests certain SBs might be more detrimental to vascular health than others but it requires further exploration as total screen time was not associated with the vascular outcomes in a cross-sectional observation in 160 children aged 6-8 y old (Veijalainen et al., 2016). Furthermore, the associations may be influenced by co-occurring behaviours or residual confounding variance from socioeconomic factors, rather than the difference SBs themselves as the sedentary stimulus on the vasculature should be the same in all SBs.

2.8 INTERACTION BETWEEN CARDIOMETABOLIC RISK, PHYSICAL ACTIVITY, SEDENTARY TIME AND VASCULAR OUTCOMES

Favourable associations between PA (especially VPA) in late adolescence (9-15 y old) and early adulthood (18-36 y old) and arterial elasticity and compliance in adulthood (20-36 y old) have been reported (Palve et al., 2014, van de Laar et al., 2011). In contrast, an unfavourable association has been found between television viewing time in adulthood (32 and 36 y old) and arterial stiffness in adulthood (36 y old) (van de Laar et al., 2014). Further analysis suggested the beneficial associations are due to simultaneous favourable associations between VPA and other traditional CMR factors (van de Laar et al., 2011). Differences in brachial and femoral compliance coefficients were explained by 28 and 62% from VPA related impacts on other CVD risk factors, but not for the distensibility coefficient. In contrast, only up to 30% of the change in arterial stiffness from television viewing could be explained by simultaneous changes in CVD risk factors (van de Laar et al., 2014). Therefore, the associations between PA and television viewing time with arterial stiffness and elasticity are partially mediated by changes in traditional CVD risk factors but there are also direct effects of PA and television time, although the mechanism of action is currently unclear.

2.9 INTERVENTIONAL EVIDENCE

2.9.1 Interventional evidence for sedentary behaviour and vascular outcomes

A single paediatric study has demonstrated the transient impairment in endothelial function of the lower limb measured using FMD (Figure 2.4) from 7.04 ± 0.30% (mean ± standard error of the mean) to 4.71 ± 0.20% following an acute bout of 3 h of uninterrupted sitting (a type of SB) in pre-pubertal girls (McManus

et al., 2015). Numerous adult studies have reported similar findings within 3-6 h of uninterrupted sitting (Thosar et al., 2014, Restaino et al., 2015, Thosar et al., 2015, Restaino et al., 2016, Vranish et al., 2017) which support this paediatric observation. The adult literature has also demonstrated that this response is limb specific, with the transient impairment in endothelial function only observed in the lower limb and not in the brachial artery (Thosar et al., 2014, Restaino et al., 2015). The limb specific response to uninterrupted sitting observed in adults may explain the absence of a statistically significant association between ST and brachial artery FMD in children observed by Hopkins et al. (2012). The mechanism by which this impairment in endothelial function occurs is from a reduction in shear stress in adults (Restaino et al., 2016), but the mechanism has not yet been investigated in a paediatric population.

Adult literature has also provided insight into the time course of the response, with evidence showing the largest decline in function (FMD) occurs in the first h (Thosar et al., 2014, Thosar et al., 2015), and an additional study has shown that 10 min of uninterrupted sitting was insufficient to result in a decline in endothelial function (Vranish et al., 2018). To date, there is no consensus in the adult literature on whether there are sex differences present in the response, with only males experiencing the decline in endothelial function (Vranish et al., 2017) or not (O'Brien et al., 2019). In part this is due to a lack of studies including female participants, and also specifically examining the question of whether there are sex differences in the response. At present, it is unknown if there are differences between the sexes in children and adolescents because it has only been investigated in girls so far (McManus et al., 2015).

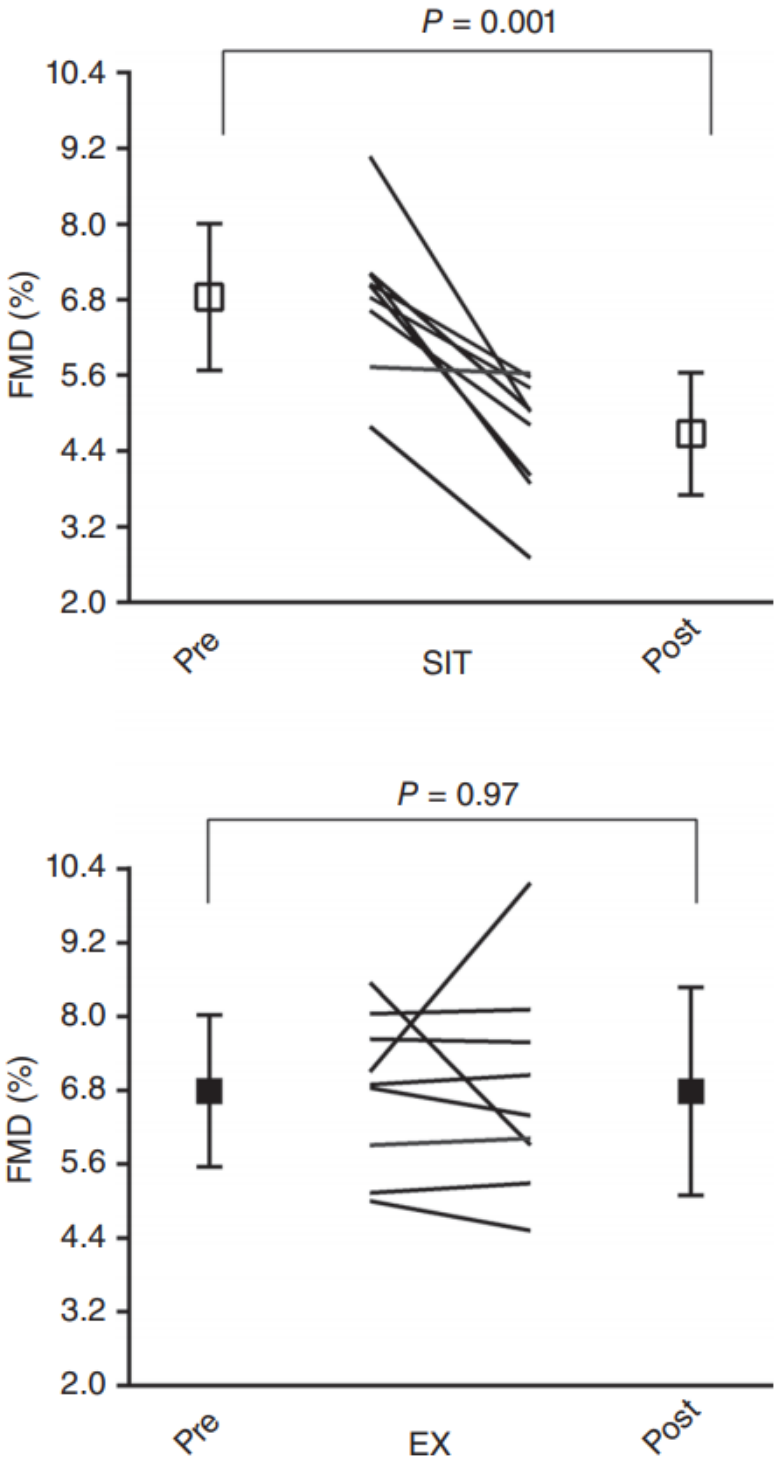

Figure 2.4 – Superficial femoral artery endothelial function (flow mediated dilation; FMD %) in 7-10 year old girls following 3 hours of uninterrupted sitting and interrupting the sitting for 10 minutes of moderate intensity cycling each hour. Reproduced with permission from McManus et al. (2015).

It is hypothesised that over time, continued exposure to impairments in vascular function would lead to remodelling of the vascular structure and consequently increased CVD risk (Thijssen et al., 2011a). However, the acute laboratory-based studies are low in ecological validity because children and adolescents are unlikely to sit uninterrupted for three h with no leg movement. Typical uninterrupted bout durations of 7 and 15 y old children and adolescents have been reported at 3.4 ± 3.8 min to 7.3 ± 3.9 min, respectively (Janssen et al., 2016). Contemporary data also have also shown children and adolescents (*n*=78; 6-17 y old) sit uninterrupted for periods of up to 30 min and engage in at least one bout of uninterrupted sitting lasting between 30 min to 1 h (Tallon et al., 2021).

2.9.2 Interventional evidence for the benefits of physical activity breaks in sedentary time on vascular outcomes

Interventional research indicates that PA breaks in ST may attenuate the acute negative effects of ST on endothelial function. For example, interrupting the 3 h sitting period with 10 min of moderate intensity (90% of the gas exchange threshold) cycling exercise each h prevented the transient decline in endothelial function (Figure 2.4) in pre-pubertal girls (McManus et al., 2015).

Early adult literature demonstrated that the decline in endothelial function of the popliteal artery from six h of uninterrupted sitting could be restored after 10 min of walking (~1000 steps) at a self-selected pace (Restaino et al., 2015). Other research groups have begun to investigate the impact of interrupting the sitting period with fidgeting (Morishima et al., 2016), low intensity walking (Thosar et al., 2015) and callisthenic exercises (Carter and Gladwell, 2017) which have all

prevented the decline in endothelial function in conduit arteries. Additionally, moderate (65% maximal oxygen uptake or a rating of perceived exertion of 11-13 on the 6-20 Borg scale), and high-intensity (85-95% of peak heart rate) exercise bouts prior to uninterrupted sitting have also prevented a sitting-induced decline in vascular function (Ballard et al., 2017, Morishima et al., 2017, Garten et al., 2019). Most of this research has been conducted with ostensibly healthy young adult males (<40 y old), with only three studies including females (Morishima et al., 2016, Morishima et al., 2017, Carter and Gladwell, 2017). Therefore, research needs to be expanded to include more females and also clinical groups.

Not all interventions have proven effective in preventing the decline in endothelial function with Kruse et al. (2018) reporting interrupting four h of sitting with four bouts of either 10 min standing or light intensity desk pedalling was inadequate to attenuate the sitting-induced endothelial dysfunction in the popliteal artery of overweight/obese middle aged participants. This might have been because the participants were older (38 ± 3 y) than in the previous studies and these individuals were also habitually sedentary so may require a larger PA stimulus than was provided to elicit a beneficial vascular response. In a recent study, no significant decline in superficial femoral artery endothelial function was seen from uninterrupted sitting but the use of less frequent but longer duration walking breaks (8 min every 2 h) was able to prevent the decline in blood flow compared to shorter, more frequent breaks (2 min every 30 min) (Carter et al., 2019). Therefore, the duration and number of PA breaks might be important factors to consider to prevent the decline in endothelial function from uninterrupted sitting, suggesting that the patterning of PA and ST is important. Together, these data

highlight a complex link between ST and PA breaks which requires further study in paediatric populations to inform development of evidence-based PA and ST guidelines to maximise vascular health benefits.

2.9.3 Likely mechanism for how activity breaks maintain endothelial function

Although the mechanism of impairment in endothelial function from uninterrupted sitting is currently unclear in children and adolescents, the data in adults indicated that it is caused by a reduction in shear stress (Restaino et al., 2016). Therefore, strategies which increase or maintain shear stress (e.g. exercise or PA) may prove effective to maintain endothelial function during uninterrupted sitting.

2.10 COMPOSITIONAL DATA ANALYSIS

2.10.1 The use of compositional data analysis for physical activity time data

As discussed in 2.3.1, PA time data is compositional in nature (Dumuid et al., 2018). This means that the component parts (LPA, MVPA, ST etc.) sum to make a whole such as a 24 h day or the waking hours. By increasing the amount of time spent in one activity behaviour (e.g. MVPA), the amount of time spent in at least one of the other activity behaviours (e.g. LPA and/or ST) must be reduced because the sum of the component parts cannot be increased. Therefore, PA time data needs to be analysed in a way that reflects this. Compositional data also has a different sample space from the real space associated with unconstrained vectors (Aitchison, 1982b) so it cannot be represented appropriately in the standard three-dimensional Cartesian co-ordinates system (Figure 2.5.a). For example, in Figure 2.5.a the points A, B and C imply that you could increase the proportion of time spent in MVPA while keeping the time spent

in LPA and ST the same which is not possible and in fact, point C is not attainable because it exceeds 100% of time spent in MVPA. Instead, compositional data are better depicted in either a constrained simplex space (Figure 2.5.b), or in a ternary plot (Figure 2.5.c). A constrained space means the space is closed to a defined total, therefore a change in one of the activity behaviours, changes one or more of the remaining behaviours to ensure the total remains the same. The ternary plot is a two-dimensional representation of the simplex, with each side of the triangle being used as an axis and each vertex representing an activity behaviour. Points which lie closer to a vertex, spend a greater proportion of time in that behaviour and if a point were to be in the centre of the triangle, it would have an equal percentage of time in all three behaviours e.g. 33.3% of the day in each activity behaviour. Point A in Figure 2.5.c represents a composition that spends a greater proportion of time in SB compared to Point B, whereas Point B represents a composition that spends a greater proportion of time in LPA and MVPA than Point A. Consequently, the mathematical properties of such data need to be taken into account in the analysis (Pedisic et al., 2017). Non-compositional multiple linear regression models are unable to account for these differing properties so the use of compositional data analysis (CoDA) has been recommended when examining physical behaviours as time spent in absolute intensity categories because it treats the compositional variables as log ratios (Dumuid et al., 2018). CoDA can also be combined with iso-temporal substitution analysis to perform compositional iso-temporal substitution analysis to understand how associations with health outcomes change when defined minutes of an activity behaviour (e.g. 15 min of MVPA) are swapped with a single behaviour (e.g. 15 min of ST) or with multiple behaviours (e.g. 7.5 min of ST and 7.5 min of LPA) (Dumuid et al., 2019).

To date, CoDA has not been used to analyse the activity data when investigating associations between ST and PA with vascular outcomes which could have produced misleading results in the existing literature. The non-compositional multiple linear regression models are unable to include all activity behaviours and wear time in the model due to the aforementioned collinearity issue (Pedisic, 2014). Therefore, the reported associations in the non-compositional multiple linear regressions are confounded by the activity behaviour that is not included as a covariate which likely inflated or diminished the strength of the association of the activity behaviour of interest with the outcome.

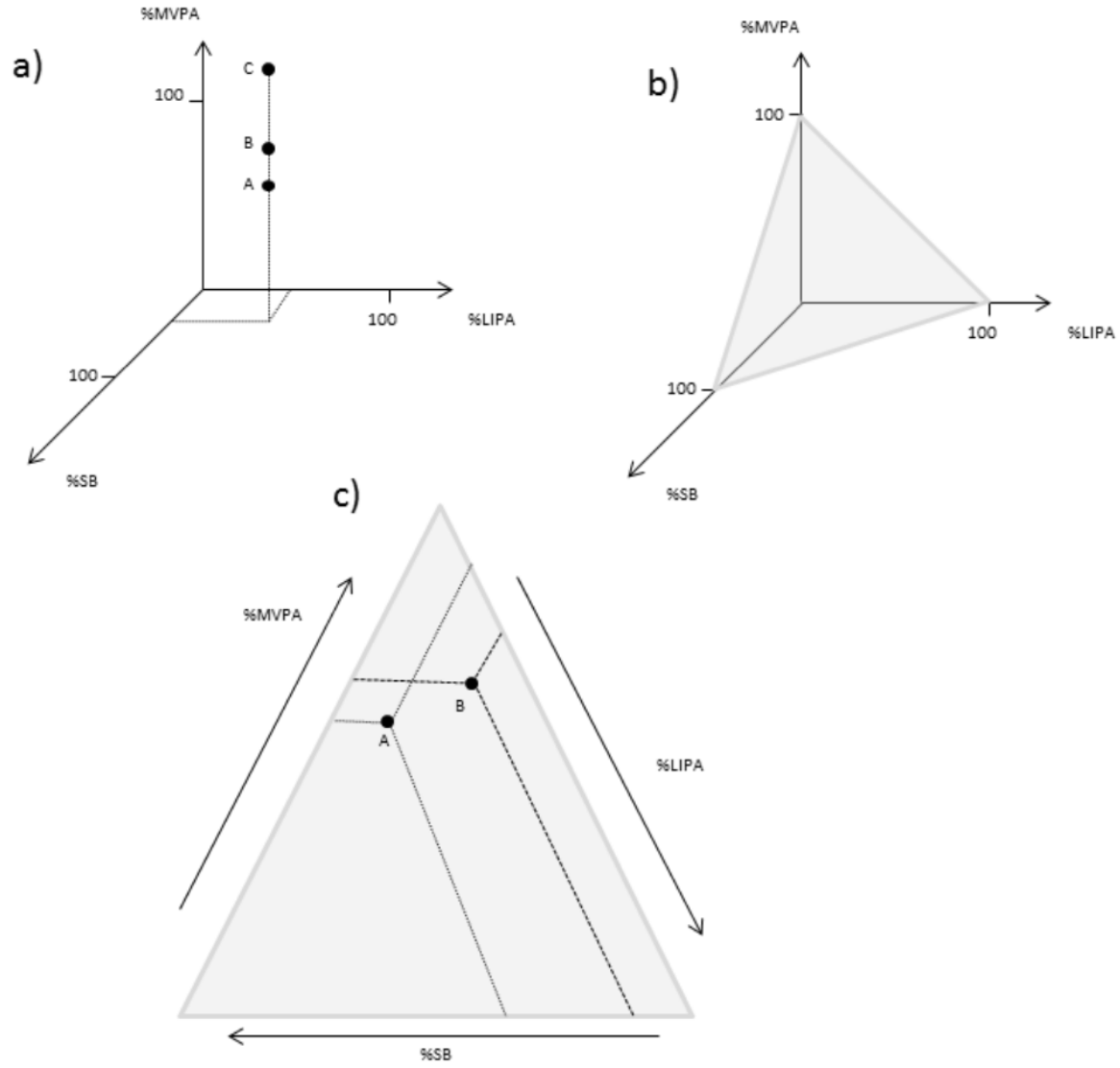

Figure 2.5 – SB = sedentary behaviour; LIPA = light intensity physical activity and MVPA = moderate to vigorous physical activity. a) activity data represented in standard three-dimensional Cartesian co-ordinates; b) activity data in a constrained simplex space and c) ternary plot to show the proportion of time of the waking day spent in each activity behaviour. Reproduced with permission from the supplementary file from Chastin et al. (2015).

The interventional evidence suggests that MPA may moderate the effect that ST has on endothelial function in prepubertal girls (McManus et al., 2015). This provides support for the need to consider the influence of the other activity

behaviours when examining the association between one of the activity behaviours on the vascular outcomes.

2.10.2 Physical activity, sedentary time and cardiometabolic risk using compositional data analysis in children and adolescents

There are limited paediatric data examining associations between PA behaviours using CoDA and only individual cardiometabolic risk factors rather than a clustered risk score. An early paper using 24-h data from children and adolescents (aged 6-17 y) and a 4-part composition (sleep, ST, LPA, and MVPA) found that relative to the other behaviours, ST was positively associated with waist circumference and BMI z-score but negatively associated with aerobic fitness (measured via the Modified Canadian Aerobic Fitness Test (Stephens and Craig, 1985, CSEP, 2003)), and LPA was positively associated with BMI z-score, waist circumference and SBP (Carson et al., 2016b). All these associations are unfavourable for health. In contrast, MVPA was positively associated with aerobic fitness and negatively associated with BMI z-score, waist circumference, SBP, C-reactive protein, plasma insulin. Sleep was also negatively associated with BMI z-score, waist circumference and SBP. These associations are all favourable for health. Carson et al. (2019) used a 4-part composition for the waking day behaviours (ST, LPA, MPA & VPA) of children and adolescents (aged 6-17 y) and found ST was also positively associated with waist circumference, and LPA was negatively associated with DBP. All these associations are unfavourable for health. Interestingly, they found VPA was negatively associated with BMI z-score and waist circumference but positively associated with HDL-cholesterol. The three associations are all favourable for health. No associations with MPA remained significant after covariate adjustment. A recent paper utilised a 4-part

composition (sleep, ST, LPA & MVPA), and found ST (relative to remaining behaviours) was positively (unfavourably) associated with a composite metabolic syndrome score in 12 y old children but not with BMI or DBP after a Holm Sequential Bonferroni adjustment (Matriccaini et al., 2021). Furthermore, MVPA was inversely (favourably) associated with BMI, DBP, the composite metabolic syndrome score and ApoB/A1 but not with glycoprotein acetyls (a novel inflammatory biomarker) after a Holm Sequential Bonferroni adjustment. Sleep was inversely associated with BMI and the composite metabolic syndrome score, but LPA was not associated with BMI after the Holm Sequential Bonferroni adjustment. Collectively, these studies demonstrate associations between MVPA and favourable markers of CMR whereas ST are associated with unfavourable indicators of CMR in line with findings of the non-compositional multiple linear regression approach.

2.10.3 Compositional data analysis compared to non-compositional multiple linear regression models

A single study in adults (21-64 y olds from the National Health and Nutrition Examination Survey; *n*= 1,937) has analysed the data using both CoDA models and non-compositional multiple linear regression models (Chastin et al., 2015). A 4-part composition was used with LPA, MVPA and ST measured via an Actigraph accelerometer and sleep was self-reported. MVPA (relative to the remaining behaviours) was inversely associated with BMI, waist circumference and C-reactive protein but positively associated with HDL concentration. All associations for MVPA are favourable for health. LPA (relative to the remaining behaviours) was inversely associated with TAG, plasma insulin and the homeostatic model of insulin resistance but positively associated with BMI. ST (relative to the remaining

behaviours) was positively associated with BMI and waist circumference. The associations for ST and LPA are all unfavourable for health. Finally, sleep (relative to the remaining behaviours) was inversely associated with BMI and DBP but positively associated with TAG, C-reactive protein and the homeostatic model of insulin resistance, which are all unfavourable for health. In the non-compositional models, ST was positively associated with BMI, waist circumference, TAG, insulin and the homeostatic model assessment of insulin resistance but inversely associated with HDL. All the ST associations are unfavourable for health. Both LPA and MVPA were inversely associated with waist circumference, TAG, insulin and homeostatic model assessment of insulin resistance. All the associations are favourable for health. In addition, MVPA was inversely associated with BMI and positively associated with HDL. Both associations are favourable for health. Sleep was positively associated with TAG and C-reactive protein but inversely with BMI and DBP. Two of these associations are favourable for health (BMI and DBP) and two are unfavourable (TAG and C-reactive protein). Table 2.1 presents a summary of how the associations between the activity behaviours with the individual CMR factors differ between the two analytical approaches. Currently, no paediatric study has presented the results using both of the analytical approaches.

Table 2.1 – Differences in the associations between activity behaviours using compositional data analysis and non-compositional multiple linear regression models with individual cardiometabolic risk factors reported by Chastin et al. (2015)

	Sleep		ST		LPA		MVPA	
	C	NC	C	NC	C	NC	C	NC
BMI	-	-	+	+	+		-	-)
C-reactive protein	+	+					-	
DBP	-	-						
Homeostatic model assessment of insulin resistance	+			+	-	-		-
HDL				-			+	+
Insulin				+	-	-		-
TAG	+	+		+	-	-		-
Waist circumference			+	+		-	-	-

C = compositional data analysis model; NC = non-compositional multiple linear regression model. (+) = significant positive association; (-) = significant negative association. BMI = body mass index; DBP = diastolic blood pressure; HDL = high-density lipoprotein; TAG = triglyceride.

2.11 THESIS AIMS

There is a paucity of high-quality paediatric data that explores the associations between device-derived PA and ST with vascular health outcomes and a clustered CMR score in a group of children that are homogenous in age. Existing data have rarely been able to account for the time spent in all the remaining activity behaviours and adjust for wear time in the analytical models because the analysis has largely been limited to multiple linear regression models that have considered ST and PA time in $min \cdot day^{-1}$ for each intensity. The use of CoDA allows for the consideration of the influence of LPA on the associations which is typically removed from non-compositional multiple linear regression models. It is important to understand the role of LPA in these associations because it is likely to be more accessible and amenable as an intervention than MVPA. Additionally,

with differences in time spent in PA and ST, the vascular outcomes and CMR between boys and girls (Steele et al., 2009, Donald et al., 2010, Stavnsbo et al., 2018), it may not be appropriate to examine these associations with the sexes combined for analysis.

The purpose of the present thesis is to utilise data from the ALSPAC study which is a large prospective cohort study from Bristol, UK (Golding et al., 2001) to:

1) Examine the associations between device-derived LPA, MVPA and ST with endothelial function, arterial elasticity, arterial stiffness and a clustered CMR score at a group level using both non-compositional multiple linear regressions and CoDA models
2) Examine the associations between the clustered CMR score with endothelial function, arterial elasticity and arterial stiffness
3) Investigate whether these associations change when controlling for known covariates – age, sex, somatic (maturation) status, mother's social class, baseline vessel diameter (when FMD was the outcome only), time between measurements, CRF scaled to lean body mass, lean mass index, clustered CMR score (apart from when CMR was the outcome), and family history of hypertension, diabetes, high cholesterol and vascular disease.
    a. In the CoDA models, all parts of the activity composition are included in the form of isometric log ratio (*ilr*) co-ordinates which also adjusts for differences in wear time between participants
    b. In the non-compositional multiple linear regression models, wear time is included as a covariate along with either MVPA or ST, but not LPA.
4) Investigate whether these associations differ with the data stratified by sex

Chapter 3

Methods

3.1 ETHICS APPROVAL AND INFORMED CONSENT

The ALSPAC Law and Ethics Committee and local NHS Research Ethics committee granted approval for the project. Written informed parental consent and child assent were obtained because participants were under the age of 16 y. Once enrolled, it was assumed participants would continue to be involved in the study unless they informed the research team otherwise. The parents were asked to give consent for the biological samples to be collected during each clinic visit but if the child subsequently refused, no further attempt was made.

3.2 PARTICIPANT RECRUITMENT

The participants were from a large prospective birth cohort study (ALSPAC) which is investigating factors that influence childhood growth and development. The full details of the study design and cohort have been published elsewhere (Golding et al., 2001). Pregnant women with a due date between April 1991 and December 1992 were eligible for the study and were approached using a variety of methods:

- Recruitment by ALSPAC study staff at routine ultrasound appointments
- Expectant mothers were sent study information with their booking information from the hospitals
- Local community midwives were asked to discuss the study at appointments with the expectant mothers
- Posters for the study were displayed in numerous locations that women were likely to attend early in pregnancy e.g., antenatal clinics, chemists, mother and toddler groups and general practitioner waiting rooms
- Through local and national press, television and radio coverage about the study

- Eligible non-enrolled mothers were approached after they had given birth in the maternity hospital by ALSPAC staff

In total, 85% of women with a due date within this period from three local health authorities in Bristol, UK were recruited, resulting in a sample size of 15,541 pregnant women. From the 15,541 pregnancies, the 14,062 liveborn children have been studied via a combination of questionnaires and clinic visits since birth and of these children, 13,988 were alive at 1 y. Approximately 10% of the sample were randomly selected from the last 6 months of the ALSPAC births (between 6th June to 11th December 1992) and invited to "Children in Focus" clinics that took place between 4 mo to 5 y. A further subsample was excluded from eligibility for the "Children in Focus" sample if the child was already part of the Avon Premature Infant Project (born <33 weeks gestation) or a full-term control. The purpose of these clinics was to allow examination of the children in more detail to gain information which could not be collected through questionnaires via the parents and to validate some parts of the questionnaires. There were additional annual clinics from age 7 to 13 y, and bi-annual clinics from 13 to 17 y that all the sample were invited to intend. A further clinic was run at age 24 y that 64% of the sample (9,997 participants) were invited to attend. To bolster the sample size after dropout, additional children were recruited after birth since the 7-y clinic. Researchers searched the child health database for children born to women resident in Avon who would have been eligible for the original study. This meant that the database was searched using the expected date of birth of the child, rather than the child's actual date of birth, to be in line with the original study criteria. Eligible participants were contacted via letters and those who agreed to join the study (*n*=1,657) were added to the cohort resulting in the final sample size of 15,645 (excluding triplet and quadruplet children).

This thesis utilises data from a number of different measurement points from the wider ALSPAC study (Table 3.1). The majority of participants had all measurements collected in one visit for each clinic but in some cases, participants were asked to return for an additional visit e.g., when there were problems with the dual x-ray absorptiometry (DEXA) scanner. For the purposes of this thesis, children with incomplete data for the vascular measurements (FMD, DC and PWV) and accelerometer variables (ST, LPA, MVPA) were excluded from the analyses, resulting in a sample of 4,277 children (2,226 girls).

Table 3.1 – Overview of what age each of the measurements were taken at that are used in this thesis

	Pre-birth	Age 9 (y)	Age 10 (y)	Age 11 (y)	Age 17 (y)
Mother's social class	X				
Anthropometrics		X	X		
Body composition via DEXA		X			
Blood outcomes		X			
Cardiorespiratory fitness		X			
Blood pressure		X	X		
Vascular outcomes			X		
Accelerometer data				X	
Family history of hypertension, diabetes, high cholesterol and vascular disease					X

DEXA = dual energy X-ray emission absorptiometry

3.3 ANTHROPOMETRY

Stature was measured to the nearest 0.1 cm (Harpenden Stadiomenter) and body mass to the nearest 0.05 kg (Tanita Body Fat Analyser; Model TBF305) during

the clinics age 9 and 10 y with the child wearing light clothing and without shoes. The child was positioned so that their heels, calves, buttocks and shoulders were in contact with the stadiometer. They were asked to keep their heels in contact with the ground and stretch up but keep their shoulders relaxed. During the measurement, the headboard was lowered until it came into contact with the child's head before a 1 kg weight was added to the headboard to minimise the effect of hair thickness.

BMI was calculated using equation 3.1 and the age and sex-specific standard deviation score (SDS) for BMI was calculated from the 1990 British Growth Reference charts (Cole et al., 1995).

$$BMI\ (kg \cdot m^2) = \frac{Body\ mass\ (kg)}{Height^2\ (m)}$$

Equation 3.1 – Body mass index

3.4 BODY COMPOSITION

A Lunar Prodigy DEXA scanner (GE Medical Systems, Chalfont St Giles, UK) was used to assess fat and lean mass at the clinic aged 9 y. Bone was also measured but for the present thesis only fat mass and lean mass are used. Body composition data assessed via DEXA are considered valid and reliable with coefficients of variation between 0.01 and 4.37% (Jaworski and Pludowski, 2013). Total body fat and lean mass indices were calculated using equation 3.2 with the height variable also taken from the clinic at 9 y. These methods of normalising were chosen to account for variation in body size between participants that could influence the measures of body composition (Wells, 2001). Additionally, the index is considered to be a more accurate measure of adiposity

and malnutrition in children, and the components of body mass (i.e., lean and fat mass) can be individually examined (Weber et al., 2012, Kakinami et al., 2014).

$$\frac{\text{Fat or lean mass (kg)}}{Height\ (m)^2}$$

Equation 3.2 – Total body fat or lean mass index

3.5 PUBERTAL STATUS

Superimposition by Translation and Rotation mixed-effects growth curve analysis (Frysz et al., 2018) was used to determine the age at peak height velocity (aPHV). This method has been shown to provide a suitable way of predicting the age at peak height velocity (Simpkin et al., 2017). Participants from the full ALSPAC cohort who had at least one measurement of stature from 5 to <10 y; 10 to <15 y and 15 to 20 years were included so that the periods of pre, circa and post PHV could be computed. In total, 5,707 participants (3,019 females) had sufficient data available. The age in years to peak height velocity (aPHV) at both the 9 and 10 y clinics are presented as an objective measure of pubertal (somatic) status.

3.6 ENDOTHELIAL FUNCTION

Six research technicians, with no prior experience, were trained over a period of five mo to undertake the vascular measurements as part of one of the routine clinic visits when participants were aged ~10 y old.

Participants rested in a supine position on a bed for 10 min in a temperature-controlled room (24-26^{o}C). High resolution (7 MHz) ultrasound (ALOKA 5500, Keymed, UK) was used to image a straight, non-branching section of the right brachial artery, 5-10 cm above the antecubital fossa. The probe was held in a

stereotactic clamp, to allow for micrometre adjustments. Baseline brachial artery diameter was recorded for 1 min before a pneumatic cuff was inflated around the forearm (immediately distal to the medial epicondyle) to 200 mmHg for 5 min. The cuff was then rapidly deflated with an automatic air regulator (Logan Research, UK) and recording continued for a further 3 min. This protocol is in line with recently updated guidelines (Doshi et al., 2001, Green, 2005, Thijssen et al., 2019). When performed in this way, FMD is considered to be nitric oxide mediated (Doshi et al., 2001, Green, 2005, Thijssen et al., 2019) but to confirm this was the case an additional measurement using a nitric oxide donor such as nitroglycerin would be required. End-diastole electrocardiogram triggered images were captured at 3 s intervals throughout the protocol. Brachial artery diameter was measured using edge-detection software (Brachial Tools, MIA, IA, USA). The FMD response was quantified using the following equation (3.3):

$$FMD\ (\%) = \frac{peak\ diameter - baseline\ diameter}{baseline\ diameter}$$

Equation 3.3 – Ratio-scaled flow mediated dilation statistic

Shear data were collected but were not available for use in the present thesis. In paediatric populations, there is no consistent significant association between FMD and shear (Thijssen et al., 2009, Bond et al., 2015b, Bond et al., 2015c, Oliveira et al., 2019, Koep et al., 2021, Kranen et al., 2021). Therefore, shear data are not reported in this thesis, nor is the FMD statistic scaled to the shear response.

3.7 ARTERIAL ELASTICITY

Brachial artery distensibility was assessed using high resolution ultrasound (ALOKA 5500, Keymed, UK) and arterial blood pressure. B-mode images of the same segment of the brachial artery used for FMD analysis were recorded for 20 s, saved onto SVHS video and transported to a laboratory in London for offline analysis (Vascular Physiology Unit, Institute of Child Health). Blood pressure was recorded simultaneously in the contralateral arm using an oscillometric blood pressure device (Omron MI-5). Arterial distension was calculated as the luminal diameter excursion from diastole to systole. The distensibility coefficient (DC, indication of vascular wall elasticity) was derived from the pulse pressure and distension of the artery and expressed as mean percentage change in cross-sectional area per unit change in blood pressure.

3.8 ARTERIAL STIFFNESS

Peak wave velocity (PWV) provided a measure of arterial stiffness. During the clinic aged 10 y, a high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, TX, USA) transcutaneously recorded pressure-pulse waveforms from the radial and carotid pulse using synchronous ECG, to provide an R-timing reference. Data were processed using integral software (SphygmoCor version 7.1, Scanmed, UK) to calculate the mean time difference between the R-wave and pressure wave on a beat-to-beat basis over 10 s. The PWV was calculated using equation 3.4:

$$PWV\ (m \cdot s^{-1}) = \frac{arterial\ path\ length\ between\ the\ carotd\ and\ radial\ arteries\ (m)}{mean\ time\ difference\ between\ R\ wave\ nad\ pressure\ wave\ (s)}$$

Equation 3.4 – Pulse wave velocity (PWV) formula

3.9 RELIABILITY OF THE ASSESSMENT MEASURES OF THE VASCULAR OUTCOMES

To assess in-house reliability, 3% of the total sample (*n*=231) were randomly selected and invited back within 6 weeks of the initial assessment for a repeat set of vascular measures, with variation in time of day, technician and position of probe in relation of the cuff. The effect of variation in skin and room temperature, time of day and consumption of caffeine and/or fried food in the two h prior to the visits were significant determinants of the vascular measures but the contribution to the variation was very small (Donald et al., 2010). Between day coefficients of variation (%) for baseline diameter and FMD (%) were 4.9 % and 10.9 % respectively (Donald et al., 2010). Additionally, reliability of the image analysis had an inter- and intra- observer variability of 1.6% and 1.2% for baseline diameter, and 5.6 % and 5.3% for FMD (%). The coefficient of variation (%) for DC was 18 % and for the arterial stiffness measure it was 8.7 %.

3.10 MEASUREMENT OF PHYSICAL ACTIVITY AND SEDENTARY TIME

Physical activity and sedentary time were assessed using accelerometry. Participants were asked to wear the accelerometers (AM7164, 2.2, ActiGraph LLC, Fort Walton Beach, FL, USA) during their waking hours on their right hip for seven consecutive days at age 11 y. The accelerometer is uniaxial and detects vertical plane acceleration and deceleration as a combined function of movement frequency and intensity. The accelerometer used a 60 s sampling period and data were expressed in counts·min^{-1}. Participants were required to remove the device for bathing or when participating in water-based activities.

Kinesoft (V.3.3.75; Kinesoft, Saskatchewan, Canada) was used to process the accelerometer data (Jago et al., 2019). A valid day was defined as a minimum of 500 min of data following the exclusion of ≥60 min of zero counts, and allowing up to two min of interruptions (Jago et al., 2019). The literature recommends a zero-count criterion for non-wear time for children of 45-60 minutes of zero-counts (Chinapaw et al., 2014, Aadland et al., 2018). Previous research has recommended a minimum wear time of 10 $h \cdot day^{-1}$ for children to achieve a good estimate of habitual PA (Corder et al., 2008, Mattocks et al., 2008). Therefore, to align the inclusion criteria closer to this, the sample in the present thesis was further restricted to participants who had an average valid wear time of 600 $min \cdot day^{-1}$. Analysis was also restricted to participants who had at least three days of valid data, but there was no specification for a certain number of weekday or weekend days required (Jago et al., 2019). The use of a three d recording period has been shown to give a good estimate of PA in children with good reliability (r = 0.7) and it reduces the number of participants excluded from the analysis (Mattocks et al., 2008).

Although there are PA cut points derived specifically for the ALSPAC sample (Mattocks et al., 2007), the threshold for MVPA (>3600 $counts \cdot min^{-1}$) is much higher than other paediatric thresholds. For example, the regularly used Evenson et al. (2008) threshold for MVPA is ≥2,296 $counts \cdot min^{-1}$. The validity of MVPA thresholds ≥3000 has been questioned by the scientific community and a study has examined the classification accuracy of 5 different sets of ActiGraph cut-points when compared to energy expenditure (via indirect calorimetry) as the criterion measure (Trost et al., 2011). Cut-points for MVPA of ≥3000 $counts \cdot min^{-}$

[1] had an increased false-negative rate, lower sensitivity and marginal classification accuracy.

The use of the Mattocks et al. (2008) thresholds may therefore result in artificially low estimates for MVPA compared to other cut-points (Loprinzi et al., 2012, Banda et al., 2016, Froberg et al., 2017). Table 3.2 presents the average ST, LPA and MVPA (min·day^{-1}) for the sample used in this thesis using both the Evenson et al. (2008) and Mattocks et al. (2008) thresholds to demonstrate how these variables differ between the two methods. Furthermore, the cut-point for ST that is used (<200 counts·min^{-1}) was not defined in the original validation study (Mattocks et al., 2007), and is instead derived from a subsequent ALSPAC paper (Mitchell et al., 2012) where the macro that was used cleaned the raw data in blocks of 200 counts·min^{-1} and meant that <200 counts·min^{-1} was the lowest threshold that could be used for ST. This threshold for ST is higher than the <100 counts·min^{-1} used in previous literature (Evenson et al., 2008, Pulsford et al., 2011). Consequently, the higher threshold may artificially inflate estimates of ST and displace some LPA data. The literature has recommended the use of the Evenson et al. (2008) thresholds in paediatric populations when using the ActiGraph accelerometers (Trost et al., 2011). Consequently, the Evenson et al. (2008) thresholds were used in this thesis to determine the number of min spent being sedentary (<100 counts·min^{-1}), in LPA (100-<2296 counts·min^{-1}) and in MVPA (≥2296 counts·min^{-1}) for each child.

Table 3.2 – Comparison of average minutes per day in each activity behaviour using the Evenson vs. Mattocks thresholds

	ST min·day^{-1}		LPA min·day^{-1}		MVPA min·day^{-1}	
	Evenson	Mattocks	Evenson	Mattocks	Evenson	Mattocks
Group	354.5 ± 72.6	426.5 ± 66.8	366.5 ± 59.5	328.0 ± 58.8	57.6 ± 29.6	23.4 ± 15.6
Boys	347.6 ± 73.6	418.3 ± 68.2	367.4 ± 59.6	335.2 ± 59.9	68.2 ± 32.3	28.7 ± 17.4
Girls	360.8 ± 71.2	434.0 ± 64.6	365.6 ± 59.4	321.4 ± 57.1	47.7 ± 22.8	18.6 ± 11.9

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

3.11 BLOOD PRESSURE

At age 9 y, participants had their blood pressure recorded in the right arm using a Dinamap 9301 Vital Signs Monitor (Morton Medical, London, United Kingdom) whilst they were in the seated position. A cut-off of an arm circumference of 25 cm was used to determine whether a paediatric or adult cuff was used. The mean of two recordings was used in analysis. Mean arterial pressure (MAP) was derived using equation 3.5:

$$MAP = DBP + \frac{1}{3}(SBP - DBP)$$

Equation 3.5 – Mean arterial pressure (MAP). SBP = systolic blood pressure; DBP = diastolic blood pressure.

3.12 CARDIORESPIRATORY FITNESS

A submaximal fitness test performed on an electronically braked cycle ergometer (Lode Rechor P) was used as an indicator of cardiorespiratory fitness measured in W at age 9 y. The submaximal test used was the PWC_{170} – the physical work capacity at a heart rate of 170 beats·min^{-1}. Participants completed a 1 min warm up before completing three, 3-min stages where the resistance was increased by

20 W each stage from 20 to 60 W, and finished with a 2-min cool down. Cadence was kept between 55-65 revolutions·min^{-1} throughout the test. Participants wore a heart rate monitor with a chest strap (Polar S180) so heart rate could be recorded every 5 s. The mean heart rate during the last min of each stage was plotted against power output at this stage. Linear regression analyses were then used to predict the workload required to elicit a heart rate of 170 beats·min^{-1} as the indicator of cardiorespiratory fitness (W). The test was considered successful if the child achieved a heart rate of ≥80 beats·min^{-1} in stage one, indicating an appropriate response to the workload, and ≥150 beats·min^{-1} in stage three. The criteria of attaining ≥150 beats·min^{-1} is approximately equivalent to a workload of >70% of maximum heart rate and was used to ensure that all children exercised at a power output that elicited a heart rate close to the predicted value of 170 beats·min^{-1}. This method of assessing cardiorespiratory fitness has criterion validity in 11-16 y olds (*n*=50), where PWC_{170} (W) was reported to show a moderate correlation with absolute peak oxygen uptake (r = 0.49-0.54; $P < 0.01$) (Bland et al., 2012).

Cardiorespiratory fitness data (W) were subsequently ratio (equation 3.6) and allometrically scaled using log-linear regression models for body mass and lean mass to control for the influence of body size and composition on cardiorespiratory fitness (Welsman et al., 1996, Loftin et al., 2016). Sex and body mass (equation 3.7) or lean body mass (equation 3.8) were entered as the independent variables and PWC_{170} (W) as the dependent variable in the regression models. The resulting scaling exponent (β) was 0.24 (95% CI = 0.20 to 0.27) for body mass and 0.59 (95% CI = 0.53 to 0.64) for lean body mass. The validity of the scaling methods to remove the influence of body size was checked

by correlating the scaled cardiorespiratory fitness values against the relevant body size variables at both a group level and with the data stratified by sex using a Pearson correlation (Table 3.3). The Pearson correlation showed the ratio scaling method failed to remove the influence of body size on cardiorespiratory fitness. The allometric scaling method performed better, although it was less effective in the boys.

$$PWC_{170}\ (W \cdot kg^{-1}) = \frac{PWC_{170}\ (W)}{total\ body\ mass\ (kg)}$$

Equation 3.6 - Ratio scaled peak work capacity at 170 beats·min^{-1} (PWC_{170}) scaled to total body mass.

$$PWC_{170}\ (W) = 3.461 + ((\ln total\ body\ mass\ (kg)) \cdot\ 0.237) - (sex\ \cdot\ 0.092)$$

Equation 3.7 – Log-linear model for allometrically scaling peak work capacity at 170 beats·min^{-1} (PWC_{170}) to body mass. Sex is coded as a categorical variable with 1 denoting male and 2 denoting female.

$$PWC_{170}\ (W) = 2.378 + ((\ln total\ body\ lean\ mass\ (kg)) \cdot\ 0.585) - (sex\ \cdot\ 0.054)$$

Equation 3.8 - Log-linear model for allometrically scaling peak work capacity at 170 beats·min^{-1} (PWC_{170}) to lean body mass. Sex is coded into a categorical variable with 1 denoting male and 2 denoting female.

Table 3.3 – Pearson correlation coefficients to assess the validity of the ratio and allometric scaling methods to control for the influence of body size and composition on cardiorespiratory fitness

	Group			Boys			Girls		
	r	r^2	*P*-value	r	r^2	*P*-value	r	r^2	*P*-value
PWC_{170}·total body mass ($W \cdot kg^{-1}$)	-0.685	0.469	**<0.001**	-0.738	0.545	**<0.001**	-0.661	0.437	**<0.001**
PWC_{170}·total body mass ($W \cdot kg^{0.237}$)	-0.035	0.001	0.11	-0.076	0.006	**0.025**	0.039	0.002	0.17
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$)	0.038	0.001	0.09	-0.081	0.007	**0.020**	0.033	0.001	0.26

PWC_{170} = peak work capacity at 170 beats per minute

3.13 BLOOD OUTCOMES

Non-fasted blood samples were collected using standardised procedures during the clinic aged 9 y. Blood samples were spun immediately after collection and stored at -80^{o}C for analysis in 2008. The median storage time of samples was 7.5 y and samples were not subject to any freeze-thaw cycles prior to analysis. Plasma lipids (total cholesterol, triglycerides [TAG], high density lipoprotein [HDL]) were analysed using enzymatic reagents (Falaschetti et al., 2010). An enzyme-linked immunosorbent assay was used to measure insulin concentration (Mercodia, Uppsala, Sweden). The coefficient of variation for all assays was <5%.

3.14 CARDIOMETABOLIC RISK SCORE

A clustered CMR score was computed rather than using individual risk factors because it is a better marker of cardiovascular health in children due to daily fluctuations in risk factors (Andersen et al., 2006). The CMR score was computed from variables assessed at age 9 y: fat mass index, MAP, TAG, total cholesterol: HDL ratio and insulin concentration. There is not a universal CMR score for paediatric research but most researchers agree that a clustered CMR score should include measures of hypertension, adiposity, dyslipidaemia and insulin resistance (Stavnsbo et al., 2018), hence why the CMR score in this thesis contains these components. Fat mass index was chosen rather than fat mass or BMI because it is a better measure of adiposity in children (Kakinami et al., 2014). All variables were log-transformed for analysis due to breaking the assumption of normality (assessed via the Shapiro-Wilk test) and the sex-specific z-scores for each variable were calculated. To compute the final CMR score, the sum of these z-scores was divided by 5 (the number of variables) in line with previous ALSPAC literature (Stamatakis et al., 2015).

3.15 FAMILY HISTORY OF DISEASE

Family history was assessed for presence of hypertension, diabetes, high cholesterol and vascular disease at age 17 y by briefly asking participants during the clinic visit.

3.16 MISSING DATA

After restricting the sample size for analysis to participants who had a complete set of vascular measures at age 10 y and accelerometery variables age 11 y, missing data (listwise deletion) were present for a number of covariates in differing amounts (Table 3.4). Little's MCAR test (performed in SPSS, v.26) showed that the data were missing at random (P<0.001) and therefore, imputation of missing data was performed using the multiple imputation by chained equations (mice) package (v 3.12.0) in RStudio (http://cran.r-project.org). It was estimated that 20 cycles of imputations with 10 iterations via regression models would be sufficient based on the percentage of missing values for variables (Newgard and Haukoos, 2007, Dangardt et al., 2019). Data from the imputation cycles was pooled for analysis. For the variable with the largest percentage of missing data (mother's social class, 54.4% missing), the estimate using Rubin's formula (Rubin, 1988), was that the pooled values were 97% efficient after 20 imputations.

After imputation, data were examined to check the imputed values maintained a similar distribution as the observed values. Table 3.5 presents the variables that were imputed along with the minimum and maximum values and table 3.6 presents summary statistics (Mean, SD, percentages) of the observed vs.

imputed values. For clarity, it is preferred that just the imputed form of the data analyses are presented in the main text (Mackinnon, 2010), therefore, the sensitivity analysis is presented in the appendix (Tables A.3 to A11, A.13 and A.14).

Table 3.4 – Listwise exclusion of missing data

Variable	Valid data available (*n*)	Missing data (%)
Age at 9-year clinic (y)	4089	4.4
Age at 10-year clinic (y)	4277	0
aPHV at 9 (y)	3370	21.2
aPHV at 10 (y)	3449	19.4
Body mass at 9 (kg)	4080	4.6
Body mass at 10 (kg)	4261	0.4
Stature at 9 (cm)	4043	5.5
Stature at 10 (cm)	4247	0.7
BMI at 10 ($kg \cdot m^{-2}$)	4240	0.9
BMI-SDS at 10	4240	0.9
Mother's social class	1950	54.4
Body composition measures at 9 year clinic		
Total body fat mass (kg)	3903	8.7
Fat mass index ($kg \cdot m^{-2}$)	3863	9.7
Total body lean mass (kg)	3903	8.7
Lean mass index ($kg \cdot m^{-2}$)	3863	9.7
Fitness measure at 9 year clinic		
PWC_{170} (W)	2084	51.3
PWC_{170}·total body mass ($W \cdot kg^{-1}$)	2081	51.3
PWC_{170}·total body mass ($W \cdot kg^{0.237}$)	2081	51.3
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$)	1991	53.4
Metabolic profile at 9 year clinic		
Cholesterol ($mmol \cdot L^{-1}$)	2778	35.0
HDL ($mmol \cdot L^{-1}$)	2778	35.0
Total cholesterol:HDL ratio	2778	35.0
TAG ($mmol \cdot L^{-1}$)	2778	35.0
Insulin ($mU \cdot L^{-1}$)	2760	35.5

Cardiometabolic risk score	2546	40.5
Family history of hypertension, diabetes, high cholesterol and vascular disease	2744	35.8
Vascular measures		
Systolic blood pressure age 9 (mmHg)	4045	5.4
Diastolic blood pressure age 9 (mmHg)	4046	5.4
MAP age 9	4045	5.4
Systolic blood pressure age 10 (mmHg)	4277	0
Diastolic blood pressure age 10 (mmHg)	4277	0
Baseline vessel diameter (mm) age 10	4277	0
FMD absolute (mm) age 10	4277	0
FMD (%) age 10	4277	0
DC (% per mmHg) age 10	4277	0
PWV ($m \cdot s^{-1}$) age 10	4277	0
Accelerometer measures at 11 year clinic		
Accelerometer wear time ($min \cdot day^{-1}$)	4277	0
ST ($min \cdot day^{-1}$)	4277	0
LPA ($min \cdot day^{-1}$)	4277	0
MVPA ($min \cdot day^{-1}$)	4277	0
Time between visits (y)	4277	0
Time between CMR score and vascular visits (y)	4089	4.4
Time between CMR score and accelerometer visits (y)	4089	4.4

aPHV = age in years from peak height velocity; BMI = body mass index; BMI-SDS = body mass index standard deviation score; PWC_{170} = peak work capacity at 170 beats per minute; HDL = high density lipoprotein; TAG = triglyceride; MAP = mean arterial pressure; FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

Table 3.5 – Variables included in the multivariable multiple imputation model

Variable	Missing values imputed?	Imputed (*n*)	Imputed values	
			Minimum	Maximum
Age at 9-year clinic (y)	Yes	188	8.8	11.6
Age at 10-year clinic (y)	NA			
Sex	NA			
aPHV at age 9 (y)	Yes	907	-7.7	0.5
aPHV at age 10 (y)	Yes	828	-6.6	1.8
Body mass age 9 (kg)	Yes	197	19.4	71.4
Body mass age 10 (kg)	Yes	16	27.9	63.8
Stature age 9 (cm)	Yes	234	118.1	162.7
Stature age 10 (cm)	Yes	30	124.5	156.3
BMI age 10 ($kg \cdot m^{-2}$)	Yes	37	13.7	29.4
BMI-SDS age 10	Yes	37	-2.0	3.2
Mother's social class	Yes	2327	1	6
Body composition measures at 9 year clinic				
Total body fat mass (kg)	Yes	374	1.43	31.93
Fat mass index ($kg \cdot m^{-2}$)	Yes	414	0.79	15.07
Total body lean mass (kg)	Yes	374	15.64	37.71
Lean mass index ($kg \cdot m^{-2}$)	Yes	414	9.88	16.95
Fitness measure at 9 year clinic				
PWC_{170} (W)	Yes	2193	36	88
PWC_{170}·total body mass ($W \cdot kg^{-1}$)	Yes	2196	0.8	3.2
PWC_{170}·total body mass ($W \cdot kg^{0.237}$)	Yes	2196	16.7	37.0
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$)	Yes	2286	5.9	13.7
Metabolic profile at 9 year clinic				
Cholesterol ($mmol \cdot L^{-1}$)	Yes	1499	2.44	7.97
HDL ($mmol \cdot L^{-1}$)	Yes	1499	0.44	2.88
Total cholesterol:HDL ratio	Yes	1499	1.35	10.00

TAG (mmol·L^{-1})	Yes	1499	0.18	4.82
Insulin (mU·L^{-1})	Yes	1517	0.20	2730.00
Cardiometabolic risk score	Yes	1731	-1.74	2.96
Family history of hypertension, diabetes, high cholesterol and vascular disease	Yes	1533	1	2
Vascular measures				
Systolic blood pressure age 9 (mmHg)	Yes	232	74	145
Diastolic blood pressure age 9 (mmHg)	Yes	231	41	79
MAP age 9	Yes	232	56	98
Systolic blood pressure age 10 (mmHg)	NA			
Diastolic blood pressure age 10 (mmHg)	NA			
Baseline vessel diameter (mm) age 10	NA			
FMD absolute (mm) age 10	NA			
FMD (%) age 10				
DC (% per mmHg) age 10	NA			
PWV (m·s^{-1}) age 10	NA			
Accelerometer measures				
Accelerometer wear time (min·day^{-1})	NA			
ST (min·day^{-1})	NA			
LPA (min·day^{-1})	NA			
MVPA (min·day^{-1})	NA			
Time between visits (y)	NA			
Time between CMR score and vascular visits (y)	Yes	188	-0.8	2.2
Time between CMR score and accelerometer visits (y)	Yes	188	0.3	3.3

aPHV = age in years from peak height velocity; BMI = body mass index; BMI-SDS = body mass index standard deviation score; PWC_{170} = peak work capacity at 170 beats per minute; HDL = high density lipoprotein; TAG = triglyceride; MAP = mean arterial pressure; FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

Table 3.6 – Participant characteristics using imputed and observed data

Variable	Group		Boys		Girls	
	Observed	Imputed	Observed	Imputed	Observed	Imputed
Age at 9-year clinic (y)	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.3
aPHV at 9 (y)	-2.8 ± 1.28	-2.8 ±1.3	-3.8 ± 0.9	-3.7 ± 1.0	-2.0 ± 0.9	-2.0 ± 0.9
aPHV at 10 (y)	-2.0 ± 1.3	-2.0 ± 1.3	-3.0 ± 0.9	-2.9 ± 1.0	-1.2 ± 0.9	-1.2 ± 0.9
Body mass at 9 (kg)	34.3 ± 7.1	34.2 ± 7.1	34.0 ± 6.7	33.9 ± 6.7	34.5 ± 7.4	34.4 ± 7.4
Body mass at 10 (kg)	37.6 ± 8.2	37.6 ± 8.2	37.2 ± 7.8	37.2 ± 7.8	38.0 ± 8.5	38.0 ± 8.5
Stature at 9 (cm)	139.3 ± 6.2	139.3 ± 6.2	139.6 ± 6.0	139.6 ± 6.0	139.0 ± 6.4	139.0 ± 6.4
Stature at 10 (cm)	143.8 ± 6.6	143.8 ± 6.6	143.7 ± 6.3	143.7 ± 6.3	143.8 ± 6.8	143.8 ± 6.8
BMI at 10 ($kg \cdot m^{-2}$)	18.1 ± 3.0	18.1 ± 3.0	17.9 ± 2.7	17.9 ± 2.9	18.2 ± 3.1	18.2 ± 3.1
BMI-SDS at 10	0.27 ±1.14	0.27 ± 1.14	0.34 ± 1.13	0.34 ± 1.13	0.20 ± 1.14	0.20 ± 1.14
Mother's social class						
I – Professional (%)	5.5	5.7	6.5	6.6	4.5	4.8
II – Managerial & technical (%)	36.1	35.8	35.4	35.1	36.6	36.4
III – Skilled, non-manual (%)	38.9	38.3	38.1	37.8	39.6	38.8
III Skilled, manual (%)	1.5	1.9	0.9	1.2	2.2	2.4
IV – Partly unskilled (%)	14.7	14.8	15.4	15.4	14.1	14.3
V – Unskilled (%)	3.3	3.6	3.6	3.8	3.1	3.3
Body composition measures at 9 year clinic						

Total body fat mass (kg)	8.32 ± 4.85	8.29 ± 4.86	7.16 ± 4.56	7.19 ± 4.56	9.38 ± 4.86	9.30 ± 4.91
Fat mass index ($kg \cdot m^{-2}$)	4.22 ± 2.30	4.20 ± 2.29	3.61 ± 2.15	3.62 ± 2.14	4.79 ± 2.28	4.73 ± 2.30
Total body lean mass (kg)	24.40 ± 3.17	24.36 ± 3.19	25.39 ± 2.88	25.26 ± 2.95	23.49 ± 3.15	23.53 ± 3.17
Lean mass index ($kg \cdot m^{-2}$)	12.53 ± 0.97	12.54 ± 1.04	12.97 ± 0.84	12.96 ± 0.93	12.12 ± 0.90	12.16 ± 0.99
Fitness measure at 9 year clinic						
PWC_{170} (W)	64 ± 9	64 ± 9	67 ± 8	65 ± 9	62 ± 9	62 ± 9
PWC_{170}·total body mass ($W \cdot kg^{-1}$)	1.9 ± 0.4	1.9 ± 0.4	2.1 ± 0.4	2.0 ± 0.4	1.8 ± 0.3	1.9 ± 0.4
PWC_{170}·total body mass ($W \cdot kg^{0.237}$)	27.8 ± 3.8	27.8 ± 3.9	29.3 ± 3.5	28.4 ± 3.8	26.8 ± 3.7	27.2 ± 3.8
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$)	10.0 ± 1.3	9.9 ± 1.4	10.3 ± 1.2	9.9 ± 1.4	9.8 ± 1.3	9.9 ± 1.4
Metabolic profile at 9 year clinic						
Cholesterol ($mmol \cdot L^{-1}$)	4.27 ± 0.63	4.21 ± 0.81	4.21 ± 0.63	4.16 ± 0.80	4.34 ± 0.63	4.26 ± 0.81
HDL ($mmol \cdot L^{-1}$)	1.41 ± 0.31	1.38 ± 0.37	1.44 ± 0.31	1.41 ± 0.38	1.37 ± 0.30	1.36 ± 0.37
Total cholesterol:HDL ratio	3.17 ± 0.79	3.18 ± 0.93	3.03 ± 0.74	3.08 ± 0.90	3.29 ± 0.81	3.27 ± 0.95
TAG ($mmol \cdot L^{-1}$)	1.12 ± 0.52	1.13 ± 0.61	1.10 ± 0.53	1.11 ± 0.61	1.13 ± 0.52	1.14 ± 0.60
*Insulin ($mU \cdot L^{-1}$)	8.03 (10.39)	8.66 (16.87)	8.01 (11.11)	8.69 (17.03)	8.03 (9.87)	8.65 (16.94)
Cardiometabolic risk score	-0.01 ± 0.61	0.06 ± 0.71	0.00 ± 0.60	0.07 ± 0.69	0.02 ± 0.61	0.04 ± 0.71

Family history of hypertension, diabetes, high cholesterol and vascular disease						
Yes (%)	30.2	30.3	29.5	29.7	30.8	30.8
No (%)	69.8	69.7	70.5	70.3	69.2	69.2
Vascular measures						
Systolic blood pressure age 9 (mmHg)	102 ± 9	102 ± 10	102 ± 9	102 ± 10	102 ± 9	103 ± 10
Diastolic blood pressure age 9 (mmHg)	57 ± 6	57 ± 6	57 ± 6	57 ± 6	58 ± 6	58 ± 6
MAP age 9	72 ± 6	72 ± 6	72 ± 6	72 ± 6	73 ± 6	73 ± 6
Time between CMR score and vascular visits (y)	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Time between CMR score and accelerometer visits (y)	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3

Data presented as mean ± SD or * indicates median (interquartile range). aPHV = age in years from peak height velocity; BMI = body mass index; BMI-SDS = body mass index standard deviation score; PWC_{170} = peak work capacity at 170 beats per minute; HDL = high density lipoprotein; TAG = triglyceride; MAP = mean arterial pressure; FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

3.17 STATISTICAL ANALYSES

Data are reported as mean ± standard deviation (SD) and β coefficients with 95% confidence intervals (CI), unless otherwise stated. Statistical significance was set at the alpha level of 0.05. Initial statistical analyses for descriptive statistics were performed using SPSS statistics (version 26, IBM, Armonk, NY) and RStudio. Data were checked for normality using the Shapiro-Wilk test and via visual inspection of histograms due to the sensitivity of the Shapiro-Wilk test with large sample sizes (Field, 2009). Mann-Whitney-U tests were used to assess sex differences in baseline characteristics for continuous variables due to the assumption of normality being violated (apart from aPHV at 9 y clinic where an independent t-test was used) and chi-squared tests for categorical variables.

3.17.1 Descriptive comparisons between the intended ALSPAC sample and the sample used in the present thesis

Descriptive comparisons between the intended ALSPAC sample and the sample used in this thesis are presented to explore how representative the sample used in the thesis was of the wider intended ALSPAC sample. Comparisons were made between the percentage of the participants who were girls vs. boys, across the mother's social class categories, vascular outcomes, and PA and ST data. It was not possible to make statistical comparisons due to some participants being part of both groups and therefore neither a paired nor independent t-test was appropriate. Therefore, the comparisons are descriptive only. The sample sizes for the intended ALSPAC sample vary for several variables due to data loss.

3.17.2 Compositional data analysis theory and approach

The principles and theory behind using the CoDA approach for analysing time spent in physical activity categories (typically sleep, sedentary time, light intensity PA and moderate-vigorous PA) have been described in detail (Chastin et al., 2015, Pedisic et al., 2017, Dumuid et al., 2018). Briefly, any composition can be defined using equation 3.9, in that the sum is the total of all the compositional parts. Equation 3.10 denotes the generic formula for a composition of waking hours and equation 3.11 gives an example of what activities this time could be comprised of in a 3-part composition.

$$\Sigma_i^d x_i = 100\%$$

Equation 3.9 – Generic formula for a composition

$$\sum_{i=1}^{d} b_i = waking\ hours$$

Equation 3.10 – Example formula for a composition of waking hours

$$t_{ST} + t_{LPA} + t_{MVPA} = waking\ hours$$

Equation 3.11 – Example 3-part composition for a waking day from an accelerometer measurement. ST = sedentary time; LPA = light physical activity; and MVPA = moderate to vigorous physical activity

To predict the value of an outcome (*Y*) based upon measured time spent in activity behaviours (A), standard linear regression models can be used whereby the original activity variables are replaced by the *ilr* co-ordinates (Hron et al., 2012). For example, if A is comprised of *d* parts, equation 3.12 describes this composition and equation 3.13 denotes the expected value of Y. The *ilr* co-

ordinates are defined using the generic formula in equation 3.14 (Chastin et al., 2015). The vector of all the *ilr*-variables can be defined using Z, where Z = (z_1, …, z_{d-1}) and consequently equation 3.13 can be replaced by equation 3.15.

$$A = \Sigma_{i=1}^{d} a_i$$

Equation 3.12 – Example formula for a composition

$$E(Y|A) = \beta_0 + \beta_1 a_1 + \cdots + \beta_d a_d$$

Equation 3.13 – Example formula to construct a linear regression from a composition

$$z_i = \sqrt{\frac{d-i}{d-i+1}}\ ln\frac{b_i}{\sqrt[d-i]{\prod_{j=i+1}^{d} b_j}}\ with\ i = 1, 2, \ldots, d-1$$

Equation 3.14 - Generic formula to define the isometric log ratios for a composition comprised of *d*-parts

$$E(Y|Z) = y_0 + y_1 z_1 + y_2 z_2 + y_3 z_3 \ldots + y_{d-1} z_{d-1} + covariates$$

Equation 3.15 – Alternative generic formula for the linear regression with isometric log ratios

The compositions (v 2.0-1) (van den Boogaart and Tolosana-Delgado, 2008) and robCompositions (v 2.3.0) (Templ et al., 2011) packages in RStudio (v 4.0.3) were used to close the activity composition to 1, to represent the total of the waking day wear time recording period for each individual. Therefore, the time spent in each behaviour is represented for each as a proportion of the whole e.g., 6 h of ST for a participant with a total waking day wear time of 12 h is represented as

0.5 or 50%. This allows for the subsequent calculation of the geometric mean and robust variation matrices. The robust variation matrix replaces the univariate standard deviation (Aitchison, 1982a, Chastin et al., 2015) and provides an idea of the spread of the compositional parts pairwise (i.e. between LPA and MVPA), where values closer to zero indicate the parts are more dependent upon each other. For reporting the waking hours in each activity, the robust mean composition was linearly adjusted to the theoretical sum of 16 waking hours per day (e.g. the 6 h of ST for a 12 h recording period representing 50% of wear time becomes 8 h of ST in the adjusted results) in line with previous literature in this age group (Gaba et al., 2020). This is because school-aged children have been reported to spend 8 h a day sleeping (Spruyt et al., 2011).

For the analyses, three sets of isometric log ratios (*ilr's*) were defined, from the generic formula in equation 3.14 and this created sets of co-ordinates comprised of two parts (one less than the number of compositional parts). Sequential binary partition (Egozcue and Pawlowsky-Glahn, 2005) makes the *ilr* co-ordinates interpretable where the first *ilr* of the set expresses the behaviour of interest in relation to the others (e.g. ST : LPA and MVPA) and the second *ilr* expresses the ratio between the two remaining behaviours (e.g. LPA : MVPA). Since all three sets or *ilr* co-ordinates describe the same sub-composition, the resulting multiple correlation coefficients would be exactly the same no matter which set of co-ordinates are entered into the model (Pawlowsky-Glahn et al., 2015). However, by using examining each set of co-ordinates individually, it allows the investigation of the association of each activity behaviour in relation to the other two parts. Therefore, the following *ilrs* were defined for ST (equations 3.16 and 3.17), MVPA (equations 3.19 and 3.20) and LPA (equations 3.22 and 3.23)

respectively and the accompanying linear regression equations are defined in equations 3.18, 3.21 and 3.23.

$$z_1 = \sqrt{\frac{2}{3}} ln \frac{ST}{\sqrt[2]{LPA \cdot MVPA}}$$

Equation 3.16 – *isometric log ratio₁* examining the association between proportion of time spent in sedentary time (ST) while controlling for time spent in light physical activity (LPA) and moderate to vigorous physical activity (MVPA)

$$z_2 = \sqrt{\frac{1}{2} ln \frac{LPA}{\sqrt[1]{MVPA}}}$$

Equation 3.17 – *isometric log ratio₂* explaining the ratio between light physical activity (LPA) and moderate to vigorous physical activity (MVPA) for examining the association between proportion of time spent in sedentary time (ST) while controlling for time spent in light physical activity and moderate to vigorous physical activity

$$E(Y|Z) = \beta_0 + \beta_1 z_1 + \beta_2 z_2 + covariates$$

Equation 3.18– linear regression formula for examining the association between the proportion of time spent in sedentary time (ST) while controlling for time spent in light physical activity (LPA) and moderate to vigorous physical activity (MVPA) on an outcome (Y)

$$z_3 = \sqrt{\frac{2}{3}} ln \frac{MVPA}{\sqrt[2]{ST \cdot LPA}}$$

Equation 3.19 – *isometric log ratio₁* examining the association between proportion of time spent in moderate to vigorous physical activity (MVPA) while controlling for time spent in sedentary time (ST) and light physical activity (LPA)

$$z_4 = \sqrt{\frac{1}{2}} ln \frac{ST}{\sqrt[1]{LPA}}$$

Equation 3.20 – *isometric log ratio₂* explaining the ratio between sedentary time (ST) and light physical activity (LPA) for examining the association between proportion of time spent in moderate to vigorous physical activity (MVPA) while controlling for time spent in sedentary time and light physical activity

$$E(Y|Z) = \beta_0 + \beta_3 z_3 + \beta_4 z_4 + covariates$$

Equation 3.21 – linear regression formula for examining the association between proportion of time spent in moderate to vigorous physical activity (MVPA) while controlling for time spent in sedentary time (ST) and light physical activity (LPA) on an outcome (Y)

$$z_5 = \sqrt{\frac{2}{3}} ln \frac{LPA}{\sqrt[2]{MVPA \cdot ST}}$$

Equation 3.22 – *isometric log ratio₁* examining the association between proportion of time spent in light physical activity (LPA) while controlling for time spent in moderate to vigorous physical activity (MVPA) and sedentary time (ST)

$$z_6 = \sqrt{\frac{1}{2}} ln \frac{MVPA}{\sqrt[1]{ST}}$$

Equation 3.23 – *isometric log ratio₂* explaining the ratio between moderate to vigorous physical activity (MVPA) and sedentary time (ST) for examining the association between proportion of time spent in light physical activity (LPA) while controlling for time spent in moderate to vigorous physical activity and sedentary time

$$E(Y|Z) = \beta_0 + \beta_5 z_5 + \beta_6 z_6 + covariates$$

Equation 3.24 – linear regression formula for examining the association between proportion of time spent in light physical activity (LPA) while controlling for time spent in moderate to vigorous physical activity (MVPA) and sedentary time (ST) on an outcome (Y)

3.17.3 Multiple linear regressions

3.17.3.1 Compositional data analysis approach

Multiple linear regression analyses were run in RStudio with either FMD, PWV, DC or CMR score as the outcome measure and the relevant pair of *ilr* co-ordinates as the predictor variable. Four regression models were created *a priori* drawing from reviews of the existing literature (Baumgartner et al., 2020, Konigstein et al., 2020) in this area (Table 3.7) and established confounding variables (Celermajer et al., 1992, Donald et al., 2010, Hopkins et al., 2015, Sletner et al., 2018, Agbaje et al., 2019). Diet was considered as an additional confounding variable but not included because the measure available was only the average number of kilocalories consumed per day rather than detail on diet quality that has been previously adjusted for e.g., soft drink consumption (Ried-Larsen et al., 2014) or saturated fat intake (Haapala et al., 2017). The models were as follows:

1) model one contained no covariates (raw, unadjusted model)

2) model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex (in whole group models only), aPHV at 10 year clinic (y; or 9 y when CMR was outcome), mother's social class (I to V), baseline vessel diameter (mm; only when FMD was the outcome) and either the time between vascular and accelerometer measurements (y) or time between CMR score and accelerometer measurements (y)

3) model three was an extension of model 2 with further adjustment for cardiorespiratory fitness scaled to lean body mass ($W \cdot kg^{0.59}$) and the lean mass index ($kg \cdot m^{-2}$)

4) model four was an extension of model 3, with further adjustment for cardiometabolic risk score (apart from when CMR score was the outcome), and family history of hypertension, diabetes, high cholesterol, and vascular disease

In the appendix (A.12), the participant characteristics of the samples used for model 4 for the imputed data (Table 4.16) and the observed data (A.11) are presented. Furthermore, A.15 presents the participant characteristics of the samples for model 3 for the imputed data (Table 4.18) and observed data (A.13).

Table 3.7 – Summary of covariates used in the existing paediatric literature examining associations between physical activityand sedentary time with vascular outcomes

Authors	Year	Statistical analysis method	Predictor	Outcome	Covariates
Abbott et al.	2002	Correlation	PA assessed via stable isotopes	FMD of the brachial artery	Age and sex
Ascenso et al.	2016	Partial correlation analysis and multiple linear regression	ST and PA via Actigraph accelerometers	Far wall cIMT of left and right common carotid artery	Age and sex in partial correlations. Moderate PA and weight in regressions
Fujiwara et al.	2017	Logistic regression analysis	Time spent in sedentary activities (indoor play, video games, watching TV, studying) measured via questionnaire	Brachial ankle PWV	N/A
Haapala et al.	2017	Regression	ST via ACTIHEART accelerometer	Arterial stiffness index	Age, sex and wear time. Models were then mutually adjusted for ST, light PA, moderate PA and vigorous PA. Additional covariates included cardiorespiratory fitness, body fat percentage, systolic blood pressure, cardiometabolic risk score, serum leptin, serum 25(OH)S, saturated fat, sucrose, sodium intake, eating all three main meals daily or birth weight
Heil et al.	2020	Multiple linear regression	PA and ST assessed via accelerometer	PWV	Model 2) BMI-SDS, age, sex, and wear time. Model 3) Amount of time spent in the other activity intensities
Hopkins et al.	2009	Regression	PA	FMD of the brachial artery	No covariates but FMD was split into tertiles

Hopkins et al.	2012	Partial correlation analysis	ST via Actigraph accelerometer	FMD of the brachial artery	Maturation (aPHV), sex, BMI, total PA and high intensity PA
Melo et al.	2015	Multiple linear regression	PA and ST via accelerometer	Far-wall cIMT of right common carotid artery	In all models, age, sex, maturity (aPHV), pulse pressure was adjusted for. Model 2) PA intensity adjusted for. Model 3) Cardiorespiratory fitness. Model 4) body composition phenotypes
Nettlefold et al.	2012	Regression	PA and ST via accelerometer	Large artery compliance	Body surface area, systolic blood pressure, BMI, sex
Pahkala et al.	2011	Regression	Leisure time PA assessed via questionnaire with the frequency, intensity and duration of activities reported via multiple choice questions	Brachial artery FMD and aortic IMT	Age, sex and for FMD only brachial artery diameter. Further adjusted for BMI, high density lipoprotein/total cholesterol, systolic or mean arterial pressure and high-sensitivity C-reactive protein
Reed et al.	2005	Correlation	PA via the physical activity questionnaire for children	Large and small arterial compliance – radial artery	N/A
Ried-Larsen et al.	2013	Regression	PA via Actigraph accelerometers	Arterial stiffness parameters of the carotid artery (distensibility coefficient, compliance coefficient, stiffness index and Young's elastic modulus) and cIMT	Sex, pubertal status and current smoking status. All models also adjusted for time spent sedentary (via accelerometers) and waist circumference
Ried-Larsen et al.	2014	Regression	PA via Actigraph accelerometers	Arterial stiffness parameters of the carotid artery	Sex, biological maturation and height at follow up. Models also adjusted for soft-drink consumption and TV viewing time and mean arterial pressure

				(distensibility coefficient, compliance coefficient, stiffness index and Young's elastic modulus) and cIMT	
Veijalainen et al.	2016	Regression	Parent-reported total sedentary time and screen-based sedentary time; min/day via questionnaire	Arterial stiffness index and reflection index by pulse contour analysis	Model 1) All possible determinants of stiffness index, reflection index, or reflection index Δ% were forced one by one with sex and age into the linear regression models; Model 2) Maximal workload per lean mass, unstructured PA, body fat percentage, sex and age; Model 3) Further adjustment for daily number of meals and snacks, total energy intake, fat intake and sodium intake per total energy intake, the cardiometabolic risk score, the components of the cardiometabolic risk score, birth weight, the parental cardiometabolic risk score, heart rate at rest, the proportion of child's current height of predicted adult height, or outdoor temperature.

ST = sedentary time; PA = physical activity; FMD = flow mediated dilation; PWV = pulse wave velocity; IMT = intima-media thickness; BMI = body mass index; aPHV = age in years from peak height velocity.

3.17.2.2 Non-compositional multiple linear regression approach

Standard multiple linear regression models were also run in RStudio, to establish whether the observed results using CoDA were simply due to the difference in the analytical approach. Either FMD, PWV, DC or the clustered CMR score was imputed as the outcome measure and the relevant activity behaviour was selected as the predictor variable (min·day^{-1}). Five models were created based upon the previous literature (Table 3.7) with model five including additional covariates not typically adjusted for in the current literature:

1. Model one contained no covariates (raw, unadjusted model)
2. Model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex (in whole group models only), age in y from peak height velocity at 10 year clinic (y; 9 y when CMR was outcome), the time between vascular and accelerometer measurement (y), and the accelerometer wear time (min·day^{-1})
3. Model three was an extension of model two with further adjustment for an additional activity variable (min·day^{-1}; MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass (W·kg$^{0.585}$)
4. Model four was an extension of model three with further adjustment for lean mass index (kg·m^{-2}) and the clustered CMR score
5. Model five was an extension of model four with further adjustment for family history of hypertension, diabetes, high cholesterol and vascular disease, and mother's social class (I to V)

3.17.3.3 Testing for an interaction effect between sex and the activity behaviours

The multiple liner regression models described in 3.17.3.1 and 3.17.3.2 were also run with the inclusion of an interaction term between sex and the activity behaviour variable to examine if there was a significant difference in the associations between the boys and the girls (Tables A.1 and A.2). There was a significant interaction in model one for MVPA and FMD in the non-compositional regression (Table A.2), however when the covariates were added this became non-significant. Therefore, the sex difference in the MVPA-FMD relationship is explained by the covariates. No other models in either the compositional or non-compositional approach had a statistically significant interaction term. However, this thesis will still present regressions with the data stratified by sex due to the behavioural and biological differences between the sexes observed (Tables 4.2 to 4.4) and to provide the data for future meta-analyses.

3.17.3.4 Associations between vascular outcomes and the cardiometabolic risk score

Additionally, separate multiple linear regression analyses were run with FMD, PWV or DC as the outcome measure and with CMR score as the predictor variable. Three regression models are presented:

1) model one contained no covariates (raw, unadjusted)

2) model two was adjusted for age at 10 y clinic (y), sex (in whole group models only), aPHV at 10 y clinic (y), mother's social class (I to V), baseline vessel diameter (mm; only when FMD was the outcome) and the time between vascular and CMR score measurements (y)

3) model three was an extension of model two with further adjustment for cardiorespiratory fitness scaled to lean body mass ($W \cdot kg^{0.585}$) and the lean mass index ($kg \cdot m^{-2}$)

3.17.3.5 Appropriateness of models

Models were checked to confirm they met the assumptions for linearity, normality and homoscedasticity of residuals by visually inspecting the residuals vs. fitted values plot, Q-Q plot, scale-location plot and residuals vs. leverage plot (Figure 3.1 and 3.2). Visual inspection of the data and plots suggested there was no requirement for a more complex model, such as including a quadratic term. Models were assessed for collinearity, with the variance inflation factors <4 for all (Alin, 2010).

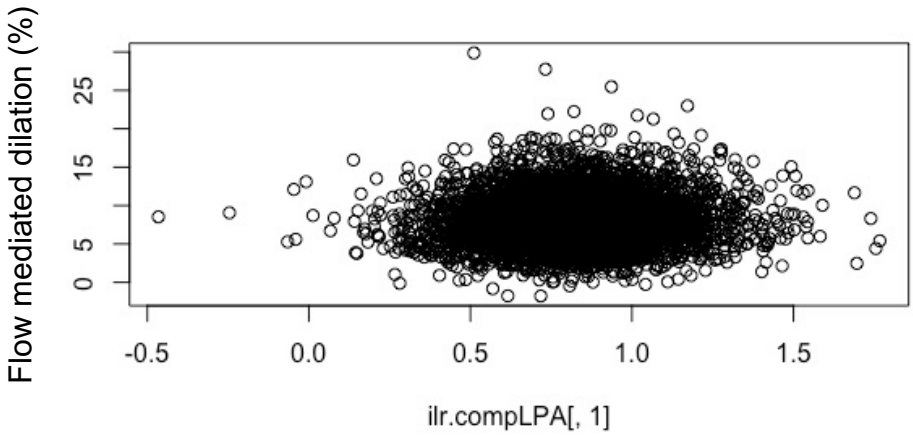

Figure 3.1 – Example scatterplot to visually examine the shape of the data. This figure shows flow mediated dilation and the isometric log ratio co-ordinate for light physical activity for the whole group.

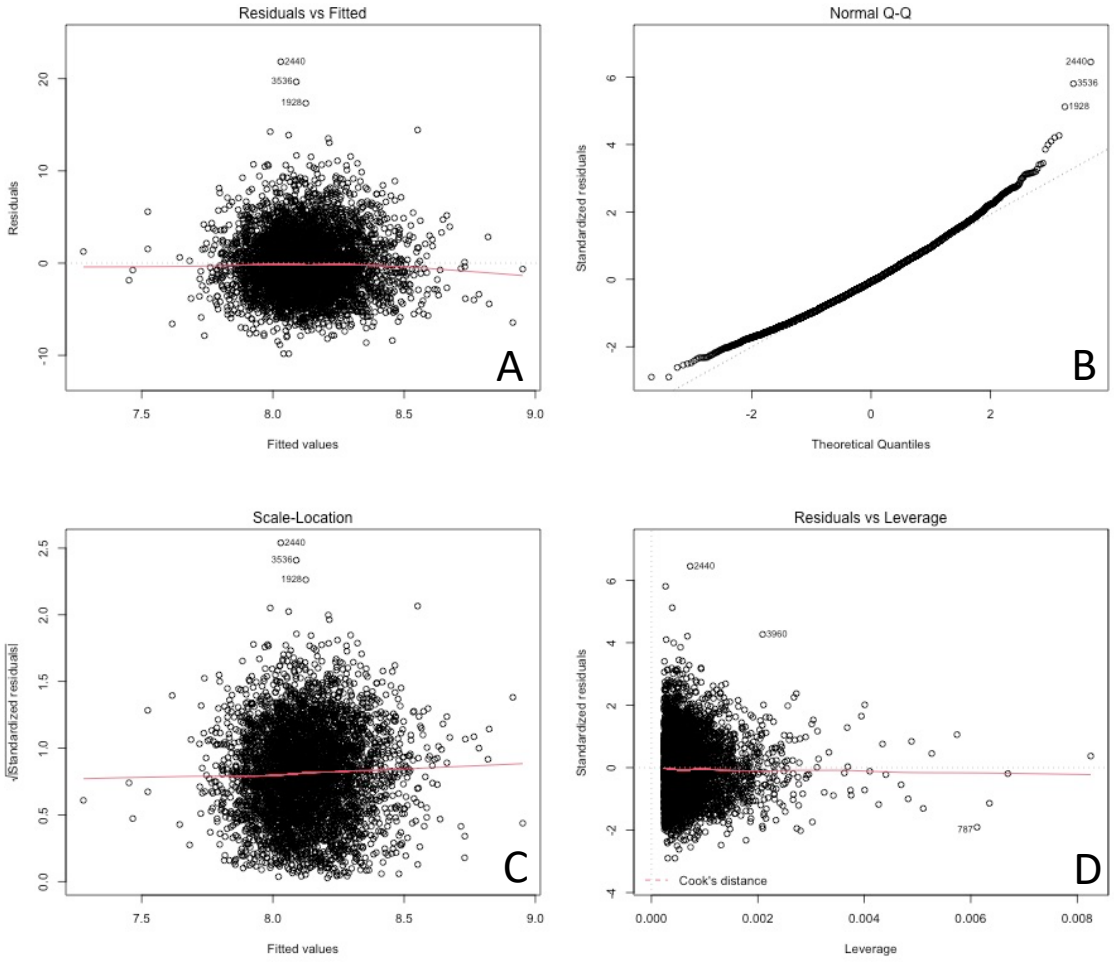

Figure 3.2 – Example of residuals vs. fitted values plot (A), Q-Q plot (B), scale-location plot (C) and residuals vs. leverage plot (D). This is model one for the compositional data analysis of light physical activity and flow mediated dilation for the whole group.

Chapter 4

Results

4.1 PARTICIPANT CHARACTERISTICS

4.1.1 Descriptive participant characteristics and confounding variables

Participant descriptive characteristics are presented in Table 4.1. The boys were taller than the girls at the 9 year clinic and had a greater BMI SD at age 10 y. In contrast, the girls were closer to aPHV than boys at both the 9 y and 10 y clinics. The girls were also heavier than the boys at the 10 y clinic and had a higher BMI at age 10 y. There were no sex differences in the age of participants at the clinics at age 9 and 10 y, body mass age 9 y, stature age 10 y, or mother's social class.

Table 4.1 – Participant descriptive characteristics for the whole group and when stratified by sex

	Group	*n*	Boys	*n*	Girls	*n*	*P*-value
Age at 9-year clinic (y)	9.8 ± 0.3	4089	9.8 ± 0.3	1959	9.8 ± 0.3	2130	0.69
Age at 10-year clinic (y)	10.6 ± 0.2	4277	10.6 ± 0.2	2051	10.6 ± 0.2	2226	0.84
aPHV at 9 y (y)	-2.8 ± 1.28	3370	-3.8 ± 0.9	1569	-2.0 ± 0.9	1801	**< 0.001**
aPHV at 10 y (y)	-2.0 ± 1.3	3449	-3.0 ± 0.9	1609	-1.2 ± 0.9	1840	**< 0.001**
Body mass at 9 y (kg)	34.3 ± 7.1	4080	34.0 ± 6.7	1954	34.5 ± 7.4	2126	0.09
Body mass at 10 y (kg)	37.6 ± 8.2	4261	37.2 ± 7.8	2044	38.0 ± 8.5	2217	**0.004**
Stature at 9 y (cm)	139.3 ± 6.2	4043	139.6 ± 6.0	1944	139.0 ± 6.4	2099	**< 0.001**
Stature at 10 y (cm)	143.8 ± 6.6	4247	143.7 ± 6.3	2040	143.8 ± 6.8	2207	0.68
BMI at 10 y ($kg \cdot m^{-2}$)	18.1 ± 3.0	4240	17.9 ± 2.7	2038	18.2 ± 3.1	2202	**< 0.001**
BMI SD at 10 y	0.27 ±1.14	4240	0.34 ± 1.13	2038	0.20 ± 1.14	2202	**< 0.001**
Mother's social class							0.06
I – Professional (%)	5.5	107	6.5	61	4.5	46	
II – Managerial & technical (%)	36.1	703	35.4	331	36.6	372	
III – Skilled, non-manual (%)	38.9	758	38.1	356	39.6	402	
III Skilled, manual (%)	1.5	30	0.9	8	2.2	22	
IV – Partly unskilled (%)	14.7	287	15.4	144	14.1	143	
V – Unskilled (%)	3.3	65	3.6	34	3.1	31	

Data presented as mean ± SD. aPHV = age in years from peak height velocity; BMI = body mass index.

4.1.2 Confounding variables

Table 4.2 presents the data on the confounding variables. The boys had more lean mass and a higher lean mass index than the girls age 9 y. Boys also had a higher PWC_{170} in absolute terms and when ratio and allometrically scaled to total body mass, and allometrically scaled to lean body mass than the girls. Furthermore, the boys had a higher HDL concentration than the girls at age 9 y. The girls had more fat mass and a higher fat mass index than the boys at age 9 y. In addition, the girls had higher cholesterol concentration, total cholesterol:HDL ratio and TAG concentration than the boys at age 9 y. There were no sex differences in insulin concentration, family history of hypertension, diabetes, high cholesterol and vascular disease, time between visits to the vascular and accelerometer clinics, time between CMR and vascular clinics, or the time between the CMR and accelerometer clinics.

Table 4.2 – Confounding variables for the whole group and when stratified by sex

	Group	*n*	Boys	*n*	Girls	*n*	*P*-value
Body composition measures at 9 year clinic							
Total body fat mass (kg)	8.32 ± 4.85	3903	7.16 ± 4.56	1870	9.38 ± 4.86	2033	**< 0.001**
Fat mass index ($kg \cdot m^{-2}$)	4.22 ± 2.30	3863	3.61 ± 2.15	1859	4.79 ± 2.28	2004	**< 0.001**
Total body lean mass (kg)	24.40 ± 3.17	3903	25.39 ± 2.88	1870	23.49 ± 3.15	2033	**< 0.001**
Lean mass index ($kg \cdot m^{-2}$)	12.53 ± 0.97	3863	12.97 ± 0.84	1859	12.12 ± 0.90	2004	**< 0.001**
Fitness measure at 9 year clinic							
PWC_{170} (W)	64 ± 9	2084	67 ± 8	866	62 ± 9	1218	**< 0.001**
PWC_{170}·total body mass ($W \cdot kg^{-1}$)	1.9 ± 0.4	2081	2.1 ± 0.4	865	1.8 ± 0.3	1216	**< 0.001**
PWC_{170}·total body mass ($W \cdot kg^{0.24}$)	27.8 ± 3.8	2081	29.3 ± 3.5	865	26.8 ± 3.7	1216	**< 0.001**
PWC_{170}·total body lean mass ($W \cdot kg^{0.59}$)	10.0 ± 1.3	1991	10.3 ± 1.2	824	9.8 ± 1.3	1167	**< 0.001**
Metabolic profile at 9 year clinic							
Cholesterol ($mmol \cdot L^{-1}$)	4.27 ± 0.63	2778	4.21 ± 0.63	1355	4.34 ± 0.63	1423	**< 0.001**
HDL ($mmol \cdot L^{-1}$)	1.41 ± 0.31	2778	1.44 ± 0.31	1355	1.37 ± 0.30	1423	**< 0.001**

Total cholesterol:HDL ratio	3.17 ± 0.79	2778	3.03 ± 0.74	1355	3.29 ± 0.81	1423	**< 0.001**
TAG (mmol·L^{-1})	1.12 ± 0.52	2778	1.10 ± 0.53	1355	1.13 ± 0.52	1423	**0.008**
*Insulin (mU·L^{-1})	8.03 (10.39)	2760	8.01 (11.11)	1348	8.03 (9.87)	1412	0.52
Cardiometabolic risk score	-0.01 ± 0.61	2546	0.00 ± 0.60	1250	0.02 ± 0.61	1296	0.24
Family history of hypertension, diabetes, high cholesterol and vascular disease							0.48
Yes (%)	30.2	830	29.5	353	30.8	477	
No (%)	69.8	1914	70.5	842	69.2	1072	
Time gap between measures							
Time between vascular and accelerometer visits (y)	1.1 ± 0.3	4277	1.1 ± 0.3	2051	1.1 ± 0.3	2226	0.53
Time between CMR score and vascular visits (y)	0.8 ± 0.3	4089	0.8 ± 0.3	1959	0.8 ± 0.3	2130	0.61
Time between CMR score and accelerometer visits (y)	1.9 ± 0.3	4089	1.9 ± 0.3	1959	1.9 ± 0.3	2130	0.38

Data presented as mean ± SD; * indicates median (interquartile range). PWC_{170} = peak work capacity at 170 beats per minute; HDL = high density lipoprotein; TAG = triglyceride.

4.1.3 Vascular outcomes

Data related to vascular outcomes are presented in Table 4.3. The boys had a larger baseline vessel diameter and a greater DC and PWV compared to the girls. In contrast, the girls had a higher DBP age 9 y, MAP age 9 y, SBP and DBP age 10 y and greater FMD (mm and %) compared to the boys. There was no sex difference for SBP at age 9 y.

Table 4.3 – Vascular measures for the whole group and when stratified by sex

	Group	*n*	Boys	*n*	Girls	*n*	*P*-value
Systolic blood pressure age 9 y (mmHg)	102 ± 9	4045	102 ± 9	1941	102 ± 9	2104	0.30
Diastolic blood pressure age 9 y (mmHg)	57 ± 6	4046	57 ± 6	1941	58 ± 6	2105	**0.014**
MAP age 9 y	72 ± 6	4045	72 ± 6	1941	73 ± 6	2104	**0.034**
Systolic blood pressure age 10 y (mmHg)	104 ± 9	4277	103 ± 9	2051	104 ± 9	2226	**0.019**
Diastolic blood pressure age 10 y (mmHg)	60 ± 8	4277	60 ± 7	2051	61 ± 8	2226	**< 0.001**
Baseline vessel diameter (mm) age 10 y	2.66 ± 0.30	4277	2.75 ± 0.29	2051	2.58 ± 0.29	2226	**< 0.001**
FMD absolute (mm) age 10 y	0.21 ± 0.08	4277	0.21 ± 0.08	2051	0.22 ± 0.08	2226	**0.039**
FMD (%) age 10 y	8.13 ± 3.39	4277	7.75 ± 3.28	2051	8.48 ± 3.45	2226	**< 0.001**
DC (% per mmHg) age 10 y	0.12 ± 0.06	4277	0.13 ± 0.06	2051	0.12 ± 0.06	2226	**< 0.001**
PWV ($m \cdot s^{-1}$) age 10 y	7.56 ± 1.23	4277	7.64 ± 1.24	2051	7.48 ± 1.20	2226	**< 0.001**

Data presented as mean ± SD. MAP = mean arterial pressure; FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient.

4.1.4 Physical activity and sedentary time data

The accelerometer data are presented in Table 4.4. The boys wore the accelerometers for longer than the girls and engaged in higher amounts of MVPA than the girls. In contrast, the girls spent more time sedentary than the boys. There were no sex differences present for time spent in LPA.

Table 4.4 – Accelerometer data for the whole group and when stratified by sex

	Group	*n*	Boys	*n*	Girls	*n*	*P*-value
Accelerometer wear time (min·day^{-1})	778.5 ± 59.6	4277	783.3 ± 60.6	2051	774.2 ± 58.4	2226	**< 0.001**
ST (min·day^{-1})	354.5 ± 72.6	4277	347.6 ± 73.6	2051	360.8 ± 71.2	2226	**< 0.001**
LPA (min·day^{-1})	366.5 ± 59.5	4277	367.4 ± 59.6	2051	365.6 ± 59.4	2226	0.24
MVPA (min·day^{-1})	57.6 ± 29.6	4277	68.2 ± 32.3	2051	47.7 ± 22.8	2226	**< 0.001**

Data presented as mean ± SD. ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

4.2 COMPARISON OF THE SAMPLE IN THIS THESIS WTH THE INTENDED SAMPLE OF THE AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN

4.2.1 Thesis sample compared to the intended Avon Longitudinal Study of Parents and Children sample

Compared to the intended ALSPAC sample (15,039 after removal of participants who withdrew consent), the sample in this thesis were more likely to be female (48.9% vs. 52.0%) and from a higher social class (Table 4.5).

Table 4.5 – Comparison of percentage of participants in each category of mother's social class between intended ALSPAC samples and the present thesis sample

Mother's social class	Intended ALSPAC sample (*n* = 4629)	Intended ALSPAC sample with one or more vascular measures at 10 y clinic (*n* = 3255)	Intended ALSPAC sample with accelerometer data at 11 y clinic (*n* = 2619)	Observed sample in this thesis (*n* = 1950)
I – Professional (%)	4.9	5.0	5.2	5.5
II – Managerial & technical (%)	33.4	36.2	36.0	36.1
III – Skilled, non-manual (%)	39.1	38.9	38.1	38.9
III Skilled, manual (%)	2.0	1.8	1.7	1.5
IV – Partly unskilled (%)	16.5	14.9	15.4	14.7
V – Unskilled (%)	4.0	3.2	3.6	3.3

N.B. The sample sizes presented here are those participants who have data available for the mother's social class variable and does not include those participants who have missing data for this variable.

4.2.2 Thesis sample compared to the intended Avon Longitudinal Study of Parents and Children sample with at least one vascular outcome

Compared to the intended sample with at least one vascular outcome (*n* = 7,444), the sample in the present thesis were more likely to be female (52.0% vs. 50.8%) but were similar in terms of the mother's social class (Table 4.5). The data were broadly similar across all the vascular outcomes between the intended ALSPAC sample who had at least one vascular outcome recorded, and the sample included in this thesis (Table 4.6).

Table 4.6 – Comparison of the vascular outcomes between the intended ALSPAC sample and the sample used in this thesis

	Intended ALSPAC sample	*n*	Thesis sample	*n*
FMD (%)	8.08% ± 3.34	6,600	8.13% ± 3.39	4277
DC (% per mmHg)	0.13 ± 0.06	6,801	0.12 ± 0.06	4277
PWV ($m \cdot s^{-1}$)	7.55 ± 1.22	7,128	7.56 ± 1.23	4277

Data presented as mean ± SD. FMD = flow mediated dilation; DC = distensibility coefficient; PWV = pulse wave velocity. N.B. although 7,444 participants in the intended ALSPAC sample had data for at least one vascular outcome, the actual available sample size in the intended ALSPAC sample is always less than 7,444 for the vascular outcomes as not all participants had valid data for all three measures due to data loss.

4.2.3 Thesis sample compared to the intended Avon Longitudinal Study of Parents and Children sample with accelerometer data

Compared to the intended sample with accelerometer data (*n* = 5,952), the sample in this thesis spent more time in MVPA but spent a similar amount of time in ST and LPA (Table 4.7). The samples were broadly similar in terms of mother's social class (Table 4.5) and percentage of female participants (52.0% in this thesis and 52.3% in the intended).

Table 4.7 - Comparison of the sedentary time and physical activity data between the intended ALSPAC sample and the sample used in this thesis

	Intended ALSPAC sample (*n*=5,952)	Thesis sample (*n*=4,277)
ST (min·day^{-1})	353.3 ± 74.6	354.5 ± 72.6
LPA (min·day^{-1})	364.7 ± 61.3	366.5 ± 59.5
MVPA (min·day^{-1})	17.0 ± 17.7	57.6 ± 29.6

Data presented as mean ± SD. ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

4.3 DESCRIPTION OF COMPOSITIONAL ACTIVITY BEHAVIOUR DATA

Compositional (geometric) means of activity behaviours are presented in Table 4.8 and the robust variation matrices are presented in Table 4.9. At group level, the greatest proportion of time was spent in LPA, followed closely by ST. The proportion of time spent in MVPA accounted for a very small part of the waking hours. When stratified by sex, boys and girls spent a similar proportion of time in LPA. However, girls spent a larger proportion of time sedentary compared to boys and consequently, boys spent a larger proportion of their time in MVPA compared to girls.

Table 4.8 – Geometric means of activity behaviours for whole group, boys and girls

	Group (*n* = 4277)		Boys (*n* = 2051)		Girls (*n* = 2226)	
	min·day^{-1}	%	min·day^{-1}	%	min·day^{-1}	%
ST	438.4	45.7	426.9	44.5	448.2	46.7
LPA	457.2	47.6	455.9	47.5	457.3	47.6
MVPA	64.5	6.7	77.2	8.0	54.4	5.7

Data are presented as the geometric mean and the percentage of the participant's waking time. ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity.

Table 4.9 – Robust variation matrices of activity behaviour components

	Group			Boys			Girls		
	ST	LPA	MVPA	ST	LPA	MVPA	ST	LPA	MVPA
ST	0.000	0.032	0.261	0.000	0.039	0.218	0.000	0.030	0.217
LPA	0.032	0.000	0.234	0.039	0.000	0.181	0.030	0.000	0.197
MVPA	0.261	0.234	0.000	0.218	0.181	0.000	0.217	0.197	0.000

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity. The robust variation matrix replaces the standard deviation and provides a spread of the compositional parts pairwise (e.g. between LPA and MVPA), where values closer to zero indicate the parts are more dependent upon each other.

4.4 ASSOCIATIONS BETWEEN SEDENTARY TIME, LIGHT PHYSICAL ACTIVITY AND MODERATE TO VIGOROUS PHYSICAL ACTIVITY WITH ENDOTHELIAL FUNCTION

4.4.1 Analyses using compositional data analysis for accelerometer data

Neither ST or LPA (relative to the other activity behaviours) were significantly associated with endothelial function in the crude or adjusted models for the whole group analysis, and when stratified by sex (Table 4.10).

The proportion of time spent in MVPA (relative to ST and LPA) was negatively associated with endothelial function in the crude model. However, following adjustment for covariates (models 2-4), the association was attenuated to null. When the analysis was stratified by sex, the proportion of time spent in MVPA (relative to ST and LPA) was not significantly associated with endothelial function in the crude or adjusted models.

Table 4.10 – Associations between sedentary time or physical activity and endothelial function using compositional data analysis for accelerometer data

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**	
	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value
Group								
ST : LPA & MVPA	0.038 (-0.331 to 0.407)	0.84	0.070 (-0.299 to 0.439)	0.70	0.084 (-0.285 to 0.454)	0.65	0.046 (-0.327 to 0.418)	0.81
LPA : MVPA & ST	0.348 (-0.124 to 0.820)	0.15	-0.050 (-0.526 to 0.427)	0.83	-0.040 (-0.515 to 0.436)	0.87	-0.026 (-0.502 to 0.449)	0.91
MVPA : ST & LPA	**-0.386 (-0.640 to -0.132)**	**0.003**	-0.020 (-0.289 to 0.248)	0.88	-0.045 (-0.314 to 0.225)	0.74	-0.019 (-0.290 to 0.252)	0.89
Boys								
ST : LPA & MVPA	-0.286 (-0.796 to 0.223)	0.27	-0.209 (-0.719 to 0.301)	0.41	-0.198 (-0.708 to 0.311)	0.44	-0.230 (-0.741 to 0.282)	0.37
LPA : MVPA & ST	0.304 (-0.362 – 0.970)	0.37	0.199 (-0.465 to 0.863)	0.55	0.223 (-0.440 to 0.887)	0.50	0.221 (-0.442 to 0.885)	0.50
MVPA : ST & LPA	-0.017 (-0.393 to 0.358)	0.93	0.010 (-0.365 to 0.386)	0.96	-0.025 (-0.403 to 0.354)	0.90	0.008 (-0.373 to 0.389)	0.97
Girls								
ST : LPA & MVPA	0.290 (-0.239 to 0.818)	0.28	0.333 (-0.197 to 0.863)	0.21	0.353 (-0.179 to 0.885)	0.18	0.305 (-0.234 to 0.844)	0.26
LPA : MVPA & ST	-0.157 (-0.840 to 0.525)	0.65	-0.274 (-0.953 to 0.406)	0.42	-0.275 (-0.954 to 0.404)	0.42	-0.248 (-0.929 to 0.433)	0.47
MVPA : ST & LPA	-0.132 (-0.521 to 0.256)	0.50	-0.059 (-0.440 to 0.322)	0.76	-0.078 (-0.460 to 0.305)	0.68	-0.057 (-0.441 to 0.327)	0.77

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class, baseline vessel diameter and the time between vascular and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

4.4.2 Analyses using a non-compositional multiple linear regression approach

Neither ST or LPA were significantly associated with endothelial function in the crude or adjusted models for the whole group analysis, or when stratified by sex (Table 4.11).

In the group analyses and for the girls, MVPA was inversely associated with endothelial function in the crude models. However, in the adjusted models, these associations were attenuated to null. For the boys, there was no significant associations between MVPA and endothelial function in either the crude or adjusted models.

Table 4.11 – Associations between sedentary time or physical activity and endothelial function using a non-compositional multiple linear regression approach

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**		**Model 5**	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
Group										
ST	1.78E-05 (-0.001 to 0.001)	0.98	2.23E-04 (-0.001 to 0.002)	0.77	3.84E-05 (-0.002 to 0.002)	0.97	-2.46E-05 (-0.002 to 0.002)	0.98	2.38E-04 (-0.002 to 0.002)	0.80
LPA	-0.001 (-0.003 to 0.001)	0.30	-1.07E-04 (-0.002 to 0.002)	0.91	-3.80E-05 (-0.002 to 0.002)	0.97	2.50E-05 (-0.002 to 0.002)	0.98	-2.38E-04 (-0.002 to 0.002)	0.80
MVPA	**-0.006 (-0.009 to -0.003)**	**0.001**	-0.001 (-0.004 to 0.003)	0.66	-0.001 (-0.005 to 0.004)	0.73	-0.001 (-0.005 to 0.004)	0.71	-0.001 (-0.005 to 0.004)	0.80
Boys										
ST	-0.001 (-0.003 to 0.001)	0.16	-0.001 (-0.003 to 0.001)	0.18	-0.001 (-0.004 to 0.001)	0.34	-0.001 (-0.004 to 0.001)	0.33	-0.001 (-0.004 to 0.002)	0.45
LPA	0.001 (-0.002 to 0.003)	0.64	0.001 (-0.001 to 0.004)	0.32	0.001 (-0.001 to 0.004)	0.34	0.001 (-0.001 to 0.004)	0.33	0.001 (-0.002 to 0.004)	0.45
MVPA	0.001 (-0.004 to 0.005)	0.79	0.001 (-0.003 to 0.006)	0.60	-3.29E-04 (-0.006 to 0.005)	0.90	-3.84E-04 (-0.006 to 0.005)	0.89	-2.80E-05 (-0.006 to 0.005)	0.99
Girls										
ST	4.73E-04 (-0.002 to 0.002)	0.65	0.002 (-0.001 to 0.004)	0.15	0.001 (-0.002 to 0.004)	0.44	0.001 (-0.002 to 0.004)	0.50	0.001 (-0.002 to 0.004)	0.39
LPA	-0.002 (-0.004 to 3.44E-04)	0.09	-0.001 (-0.004 to 0.001)	0.31	-0.001 (-0.004 to 0.002)	0.44	-0.001 (-0.004 to 0.002)	0.49	-0.001 (-0.004 to 0.002)	0.39
MVPA	**-0.007 (-0.013 to -0.001)**	**0.032**	-0.005 (-0.011 to 0.002)	0.13	-0.003 (-0.011 to 0.004)	0.37	-0.003 (-0.011 to 0.004)	0.37	-0.003 (-0.010 to 0.004)	0.40

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, baseline vessel diameter, the time between vascular and accelerometer measurement and accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

4.5 ASSOCIATIONS BETWEEN SEDENTARY TIME, LIGHT PHYSICAL ACTIVITY AND MODERATE TO VIGOROUS PHYSICAL ACTIVITY WITH ARTERIAL ELASTICITY

4.5.1 Analyses using compositional data analysis for accelerometer data

None of the activity behaviours (relative to the remaining activity behaviours) were significantly associated with arterial elasticity in the crude or adjusted models at either a whole group level, or when the analysis was stratified by sex (Table 4.12).

Table 4.12 – Associations between sedentary time or physical activity and arterial elasticity using compositional data analysis for accelerometer data

	Model 1 (crude)		Model 2		Model 3		Model 4	
	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value
Group								
ST : LPA & MVPA	-0.003 (-0.010 to 0.004)	0.36	-0.005 (-0.011 to 0.002)	0.18	-0.004 (-0.011 to 0.003)	0.21	-0.004 (-0.011 to 0.003)	0.29
LPA : MVPA & ST	0.003 (-0.006 to 0.113)	0.50	0.005 (-0.004 to 0.014)	0.26	0.005 (-0.004 to 0.014)	0.25	0.005 (-0.004 to 0.014)	0.26
MVPA : ST & LPA	1.70E-03 (-0.004 to 0.005)	0.94	-3.48E-04 (-0.005 to 0.005)	0.89	-0.001 (-0.006 to 0.004)	0.77	-0.001 (-0.006 to 0.004)	0.58
Boys								
ST : LPA & MVPA	-0.003 (-0.013 to 0.007)	0.55	-0.005 (-0.015 to 0.005)	0.33	-0.005 (-0.015 to 0.005)	0.36	-0.004 (-0.014 to 0.006)	0.39
LPA : MVPA & ST	0.003 (-0.010 to 0.015)	0.68	0.004 (-0.009 to 0.017)	0.58	0.004 (-0.009 to 0.017)	0.55	0.004 (-0.009 to 0.017)	0.50
MVPA : ST & LPA	-6.79E-03 (-0.007 to 0.007)	0.94	0.001 (-0.006 to 0.009)	0.73	0.001 (-0.007 to 0.008)	0.84	-8.61E-05 (-0.008 to 0.007)	0.98
Girls								
ST : LPA & MVPA	-0.003 (-0.012 to 0.006)	0.52	-0.004 (-0.014 to 0.005)	0.37	-0.004 (-0.014 to 0.006)	0.40	-0.003 (-0.013 to 0.007)	0.53
LPA : MVPA & ST	0.005 (-0.007 to 0.017)	0.40	0.006 (-0.006 to 0.018)	0.31	0.006 (-0.006 to 0.018)	0.31	0.006 (-0.006 to 0.018)	0.35
MVPA : ST & LPA	-0.002 (-0.009 to 0.005)	0.56	-0.002 (-0.009 to 0.005)	0.58	-0.002 (-0.009 to 0.005)	0.52	-0.003 (-0.010 to 0.004)	0.44

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class and the time between vascular and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

4.5.2 Analyses using a non-compositional multiple linear regression approach

None of the activity behaviours were significantly associated with arterial elasticity in crude or adjusted models at either a whole group level, or when the analysis was stratified by sex (Table 4.13).

Table 4.13 – Associations between physical activity or sedentary time and arterial elasticity using a non-compositional multiple linear regression approach

	Model 1 (crude)		Model 2		Model 3		Model 4		Model 5	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
Group										
ST	-6.35E-06 (-3.13E-05 to 1.86E-05)	0.62	-1.75E-05 (-4.64E-05 to 1.14E-05)	0.23	-2.46E-05 (-5.87E-05 to 9.55E-06)	0.15	-2.19E-05 (-5.61E-05 to 1.23E-05)	0.20	-2.48E-05 (-5.94E-05 to 9.80E-06)	0.15
LPA	2.32E-05 (-7.22E-06 to 5.37E-05)	0.14	2.43E-05 (-9.52E-06 to 5.81E-05)	0.15	2.46E-05 (-9.57E-06 to 5.87E-05)	0.15	2.19E-05 (-1.23E-05 to 5.61E-05)	0.20	2.48E-05 (-9.82E-06 to 5.94E-05)	0.15
MVPA	1.05E-05 (-5.07E-05 to 7.16E-05)	0.74	-7.01E-07 (-6.93E-05 to 6.79E-05)	0.98	-3.21E-05 (-1.13E-04 to 4.89E-05)	0.43	-4.46E-05 (-1.26E-04 to 3.66E-05)	0.27	-4.47E-05 (-1.26E-04 to 3.69E-05)	0.27
Boys										
ST	-7.03E-06 (-4.34E-05 to 2.93E-05)	0.71	-1.78E-05 (-5.94E-05 to 2.38E-05)	0.39	-3.08E-05 (-8.09E-05 to 1.93E-05)	0.22	-2.94E-05 (-7.95E-05 to 2.06E-05)	0.24	-3.35E-05 (-8.40E-05 to 1.70E-05)	0.19
LPA	2.46E-05 (-2.03E-05 to 6.95E-05)	0.28	2.97E-05 (-2.01E-05 to 7.95E-05)	0.23	3.08E-05 (-1.93E-05 to 8.09E-05)	0.22	2.94E-05 (-2.06E-05 to 7.95E-05)	0.24	3.35E-05 (-1.70E-05 to 8.40E-05)	0.19
MVPA	-1.85E-05 (-1.01E-04 to 6.43E-05)	0.66	-1.22E-05 (-1.00E-04 to 7.57E-05)	0.78	-4.85E-05 (-1.54E-04 to 5.73E-05)	0.36	-6.44E-05 (-1.71E-04 to 4.17E-05)	0.22	-6.46E-05 (-1.71E-04 to 4.23E-05)	0.23
Girls										
ST	-2.98E-06 (-3.76E-05 to 3.16E-05)	0.87	-1.72E-05 (-5.74E-05 to 2.29E-05)	0.39	-1.78E-05 (-6.46E-05 to 2.91E-05)	0.45	-1.43E-05 (-6.13E-05 to 3.26E-05)	0.54	-1.61E-05 (-6.37E-05 to 3.15E-05)	0.50
LPA	2.14E-05 (-2.00E-05 to 6.29E-05)	0.31	1.90E-05 (-2.70E-05 to 6.50E-05)	0.41	1.77E-05 (-2.91E-05 to 6.45E-05)	0.45	1.43E-05 (-3.26E-05 to 6.12E-05)	0.54	1.61E-05 (-3.16E-05 to 6.37E-05)	0.50
MVPA	2.60E-05 (-8.17E-05 to 1.34E-04)	0.64	2.20E-05 (-9.03E-05 to 1.34E-04)	0.70	-6.10E-06 (-1.37E-04 to 1.25E-04)	0.93	-1.59E-05 (-1.48E-04 to 1.16E-04)	0.81	-1.62E-05 (-1.48E-04 to 1.16E-04)	0.81

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

4.6 ASSOCIATIONS BETWEEN SEDENTARY TIME, LIGHT PHYSICAL ACTIVITY AND MODERATE TO VIGOROUS PHYSICAL ACTIVITY WITH ARTERIAL STIFFNESS

4.6.1 Analyses using compositional data analysis for accelerometer data

At both a group level and when the analysis was stratified by sex, the proportion of time spent in ST or LPA (relative to the other activity behaviours) was not significantly associated with PWV in either the crude or adjusted models (Table 4.14).

With the whole group combined, the proportion of time spent in MVPA (relative to ST and LPA) was positively associated with arterial stiffness in the crude model. Additional adjustments for confounding variables in models 2-4, attenuated the association to null. When the analysis was stratified by sex, no significant association was observed for either the boys or the girls in either the crude or adjusted models.

Table 4.14 – Associations between sedentary time or physical activity and arterial stiffness using compositional data analysis for accelerometer data

	Model 1 (crude)		Model 2		Model 3		Model 4	
	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value
Group								
ST : LPA & MVPA	0.022 (-0.111 to 0.156)	0.75	0.059 (-0.080 to 0.199)	0.40	0.047 (-0.092 to 0.186)	0.50	0.054 (-0.086 to 0.194)	0.44
LPA : MVPA & ST	-0.131 (-0.302 to 0.040)	0.13	-0.081 (-0.260 to 0.098)	0.37	-0.088 (-0.266 to 0.090)	0.32	-0.091 (-0.269 to 0.087)	0.31
MVPA : ST & LPA	**0.109 (0.017 to 0.201)**	**0.020**	0.022 (-0.080 to 0.123)	0.67	0.041 (-0.059 to 0.142)	0.41	0.036 (-0.065 to 0.138)	0.48
Boys								
ST : LPA & MVPA	0.079 (-0.114 to 0.272)	0.42	0.109 (-0.093 to 0.311)	0.28	0.100 (-0.095 to 0.301)	0.32	0.106 (-0.095 to 0.308)	0.29
LPA : MVPA & ST	-0.214 (-0.466 to 0.039)	0.10	-0.224 (-0.487 to 0.038)	0.09	-0.234 (-0.495 to 0.027)	0.07	-0.230 (-0.492 to 0.031)	0.08
MVPA : ST & LPA	0.135 (-0.008 to 0.277)	0.06	0.115 (-0.034 to 0.264)	0.12	0.134 (-0.014 to 0.282)	0.07	0.124 (-0.025 to 0.273)	0.10
Girls								
ST : LPA & MVPA	-0.016 (-0.200 to 0.168)	0.86	0.018 (-0.174 to 0.211)	0.85	0.003 (-0.189 to 0.194)	0.98	0.009 (-0.185 to 0.202)	0.93
LPA : MVPA & ST	0.062 (-0.176 to 0.299)	0.61	0.042 (-0.204 to 0.287)	0.73	0.040 (-0.205 to 0.284)	0.74	0.036 (-0.209 to 0.281)	0.77
MVPA : ST & LPA	-0.046 (-0.181 to 0.090)	0.51	-0.060 (-0.198 to 0.078)	0.38	-0.042 (-0.181 to 0.096)	0.54	-0.044 (-0.183 to 0.095)	0.52

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class and the time between vascular and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

4.6.2 Analyses using a non-compositional multiple linear regression approach

Neither ST or LPA were significantly associated with arterial stiffness in the crude or adjusted models for the whole group analysis, or when stratified by sex (Table 4.15).

In the group analysis, MVPA was positively associated with arterial stiffness in the crude model but following adjustment for covariates, the association was attenuated to null. With the data stratified by sex, MVPA was not significantly associated with arterial stiffness in either the crude or adjusted models.

Table 4.15 – Associations between sedentary time or physical activity and arterial stiffness using a non-compositional multiple linear regression approach

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**		**Model 5**	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
Group										
ST	-1.68E-04 (-0.001 to 3.38E-04)	0.52	2.05E-04 (-3.80E-04 to 0.001)	0.48	4.38E-04 (-2.51E-04 to 0.001)	0.20	4.32E-04 (-2.57E-04 to 0.001)	0.21	4.59E-04 (-2.37E-04 to 0.001)	0.19
LPA	-3.47E-04 (-0.001 to 2.70E-04)	0.27	-4.00E-04 (-0.001 to 2.84E-04)	0.24	-4.37E-04 (-0.001 to 2.52E-04)	0.20	-4.32E-04 (-0.001 to 2.57E-04)	0.21	-4.58E-04 (-0.001 to 2.38E-04)	0.19
MVPA	**0.002 (3.22E-04 to 0.003)**	**0.014**	4.80E-04 (-0.001 to 0.002)	0.49	0.001 (-0.001 to 0.003)	0.21	0.001 (-3.83E-04 to 0.003)	0.13	0.001 (-3.72E-04 to 0.003)	0.12
Boys										
ST	2.61E-05 (-0.001 to 0.001)	0.94	2.27E-04 (-0.001 to 0.001)	0.59	0.001 (-2.98E-04 to 0.002)	0.16	0.001 (-3.11E-04 to 0.002)	0.17	0.001 (-2.50E-04 to 0.002)	0.13
LPA	-0.001 (-0.001 to 3.30E-04)	0.21	-0.001 (-0.002 to 3.56E-04)	0.20	-0.001 (-0.002 to 2.98E-04)	0.16	-0.001 (-0.002 to 3.11E-04)	0.17	-0.001 (-0.002 to 2.50E-04)	0.13
MVPA	0.001 (-0.001 to 0.003)	0.18	0.001 (-0.001 to 0.003)	0.27	0.002 (-3.18E-04 to 0.004)	0.09	0.002 (-2.03E-04 to 0.004)	0.07	0.002 (-1.82E-04 to 0.004)	0.07
Girls										
ST	1.59E-04 (-0.001 to 0.001)	0.66	1.85E-04 (-0.001 to 0.001)	0.65	1.75E-04 (-0.001 to 0.001)	0.71	1.62E-04 (-0.001 to 0.001)	0.73	1.52E-04 (-0.001 to 0.001)	0.75
LPA	-1.77E-04 (-0.001 to 0.001)	0.68	-1.92E-04 (-0.001 to 0.001)	0.68	-1.73E-04 (-0.001 to 0.001)	0.71	-1.60E-04 (-0.001 to 0.001)	0.74	-1.51E-04 (-0.001 to 0.001)	0.75
MVPA	-1.31E-04 (-0.002 to 0.002)	0.91	-3.00E-04 (-0.003 to 0.002)	0.79	-3.71E-05 (-0.003 to 0.003)	0.98	2.43E-04 (-0.002 to 0.003)	0.85	2.30E-04 (-0.002 to 0.003)	0.86

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

4.7 ASSOCIATIONS BETWEEN SEDENTARY TIME, LIGHT PHYSICAL ACTIVITY AND MODERATE TO VIGOROUS PHYSICAL ACTIVITY WITH THE CLUSTERED CARDIOMETABOLIC RISK SCORE

4.7.1 Analyses using compositional data analysis for accelerometer data

The proportion of time spent in ST (relative to LPA and MVPA) was positively associated with the CMR score in the crude and the adjusted models in both the whole group and girls' only analyses (Table 4.16). However, in the boys only analysis, the proportion of time spent in ST (relative to LPA and MVPA) was not significantly associated with the CMR score in either the crude or adjusted models.

The proportion of time spent in LPA (relative to MVPA and ST) was not significantly associated with the CMR score at either a whole group level, or when the analysis was stratified by sex in either the crude or adjusted models.

At the group level, the proportion of time spent in MVPA (relative to ST and LPA) was not significantly associated with the CMR score in the crude model. After adjustments for covariates in models 2-4, there was an inverse association between MVPA and the CMR score. When the analysis was stratified by sex, the boys demonstrated an inverse association between the proportion of time spent in MVPA (relative to ST and LPA) and the CMR score in the crude and adjusted models. For the girls, there was an inverse association between the proportion of time spent in MVPA (relative to ST and LPA) and the CMR score in the crude model. After adjustments for covariates in model two, the association was attenuated to null but following further adjustments in models three and four, there

was an inverse association between the proportion of time spent in the proportion of time spent in MVPA (relative to ST and LPA) and the CMR score in girls.

Table 4.16 – Associations between sedentary time or physical activity and the clustered cardiometabolic risk score using compositional data analysis for accelerometer data

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**	
	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value	b *ilr1* (95% CI)	*P*-value
Group								
ST : LPA & MVPA	**0.152 (0.024 to 0.279)**	**0.021**	**0.129 (0.018 to 0.240)**	**0.023**	**0.138 (0.027 to 0.248)**	**0.015**	**0.136 (0.026 to 0.246)**	**0.016**
LPA : MVPA & ST	-0.048 (-0.181 to 0.085)	0.47	-0.021 (-0.148 to 0.106)	0.74	-0.013 (-0.138 to 0.113)	0.84	-0.010 (-0.135 to 0.115)	0.88
MVPA : ST & LPA	-0.103 (-0.220 to 0.013)	0.09	**-0.108 (-0.183 to -0.033)**	**0.005**	**-0.108 (-0.184 to -0.033)**	**0.001**	**-0.126 (-0.202 to -0.050)**	**0.001**
Boys								
ST : LPA & MVPA	0.088 (-0.066 to 0.242)	0.26	0.069 (-0.079 to 0.217)	0.35	0.075 (-0.072 to 0.222)	0.31	0.072 (-0.074 to 0.219)	0.32
LPA : MVPA & ST	0.075 (-0.110 to 0.261)	0.42	0.061 (-0.135 to 0.257)	0.54	0.070 (-0.125 to 0.265)	0.48	0.072 (-0.122 to 0.266)	0.46
MVPA : ST & LPA	**-0.163 (-0.271 to -0.055)**	**0.003**	**-0.130 (-0.239 to -0.021)**	**0.019**	**-0.145 (-0.256 to -0.034)**	**0.011**	**-0.144 (-0.255 to -0.034)**	**0.011**
Girls								
ST : LPA & MVPA	**0.219 (0.065 to 0.374)**	**0.006**	**0.189 (0.048 to 0.330)**	**0.008**	**0.201 (0.061 to 0.341)**	**0.005**	**0.199 (0.060 to 0.339)**	**0.005**
LPA : MVPA & ST	-0.111 (-0.277 to 0.055)	0.18	-0.097 (-0.260 to 0.066)	0.24	-0.093 (-0.253 to 0.068)	0.25	-0.089 (-0.250 to 0.071)	0.27
MVPA : ST & LPA	**-0.108 (-0.212 to -0.005)**	**0.038**	-0.092 (-0.191 to 0.008)	0.07	**-0.108 (-0.209 to -0.007)**	**0.034**	**-0.110 (-0.211 to -0.009)**	**0.032**

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 9 year clinic, sex (in whole group models only), age in years from peak height velocity at 9 year clinic, mother's social class and the time between cardiometabolic risk assessment and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, and family history of hypertension, diabetes, high cholesterol and vascular disease

4.7.2 Analyses using a non-compositional multiple linear regression approach

In the group analysis, ST was positively associated with the CMR score in the crude model and model two but after further adjustment for additional covariates, the associations were attenuated to null (Table 4.17). For the girls only analyses, ST was positively associated with the CMR score in the crude model and model two. Further adjustment for covariates in models three and four attenuated this association to null, however, inclusion of mother's social class and family history of hypertension, diabetes, high cholesterol and vascular disease as covariates, resulted in a positive association. A 15-min increase in ST is associated with a 0.015 increase in the CMR z-score for the girls. For the boys, ST was not significantly associated with the CMR score in either the crude or adjusted models.

For girls, LPA was inversely associated with the CMR score in the crude model and model two, but the association was attenuated to null after adjustment for additional covariates in models three and four. Inclusion of mother's social class and family history of hypertension, diabetes, high cholesterol and vascular disease as covariates, resulted in a positive association. A 15-min increase in LPA is associated with a 0.015 decrease in the z-score for the girls. In the group and boys analyses, LPA was not significantly associated with the CMR score in either the crude or adjusted models.

In the group analysis, MVPA was not significantly associated with the CMR score in the crude model but following adjustment for covariates, the association was negative. A 15-min increase in MVPA is associated with a 0.030 decrease in the

z-score for the whole group. With the data stratified by sex, MVPA was inversely associated with the CMR score in the crude model and model two for both the boys and girls. However, after further adjustment for additional covariates in models 3-5, the association was attenuated to null.

Table 4.17 – Associations between sedentary time or physical activity and the clustered cardiometabolic risk score using a non-compositional multiple linear regression approach

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**		**Model 5**	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
Group										
ST	**0.001 (2.07E-04 to 0.001)**	**0.004**	**0.001 (1.71E-04 to 0.001)**	**0.009**	3.87E-04 (-1.78E-04 to 0.001)	0.17	4.06E-04 (-1.53E-04 to 0.001)	0.15	4.38E-04 (-1.25E-04 to 0.001)	0.12
LPA	-0.001 (-0.001 to 8.71E-05)	0.09	-0.001 (-0.001 to 6.98E-05)	0.08	-3.87E-04 (-0.001 to 1.78E-04)	0.18	-4.06E-04 (-0.001 to 1.54E-04)	0.15	-4.38E-04 (-0.001 to 1.26E-04)	0.12
MVPA	-0.002 (-0.004 to -1.99E-05)	0.05	**-0.002 (-0.003 to -0.001)**	**0.001**	**-0.001 (-0.002 to -7.58E-05)**	**0.035**	**-0.001 (-0.003 to -3.03E-04)**	**0.013**	**-0.002 (-0.003 to -3.26E-04)**	**0.012**
Boys										
ST	4.93E-04 (-2.89E-05 to 0.001)	0.06	4.75E-04 (-1.10E-04 to 0.001)	0.11	9.31E-05 (-0.001 to 0.001)	0.81	1.09E-04 (-0.001 to 0.001)	0.77	1.28E-04 (-0.001 to 0.001)	0.75
LPA	-3.22E-04 (-0.001 to 0.001)	0.45	-1.87E-04 (-0.001 to 0.001)	0.62	-9.30E-05 (-0.001 to 0.001)	0.81	-1.09E-04 (-0.001 to 0.001)	0.77	-1.28E-04 (-0.001 to 0.001)	0.75
MVPA	**-0.002 (-0.003 to -0.001)**	**0.003**	**-0.002 (-0.003 to -2.91E-04)**	**0.015**	-0.001 (-0.003 to 1.68E-04)	0.08	-0.002 (-0.003 to 4.37E-05)	0.06	-0.002 (-0.003 to 4.10E-05)	0.06
Girls										
ST	**0.001 (2.98E-04 to 0.001)**	**0.002**	**0.001 (2.25E-04 to 0.002)**	**0.009**	0.001 (-5.57E-05 to 0.001)	0.07	0.001 (-2.26E-05 to 0.001)	0.06	**0.001 (4.11E-05 to 0.001)**	**0.035**
LPA	**-0.001 (-0.002 to -1.11E-04)**	**0.027**	**-0.001 (-0.002 to -7.90E-05)**	**0.029**	-0.001 (-0.001 to 5.59E-05)	0.07	-0.001 (-0.001 to 2.27E-05)	0.06	**-0.001 (-0.001 to -4.09E-04)**	**0.035**
MVPA	**-0.003 (-0.005 to -0.001)**	**0.013**	**-0.002 (-0.004 to -4.49E-04)**	**0.014**	-0.001 (-0.003 to 0.001)	0.17	-0.002 (-0.004 to 2.63E-04)	0.09	-0.002 (-0.004 to 3.02E-04)	0.09

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 9 year clinic, sex (in whole group models only), age in years from peak height velocity at 9 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

4.8 ASSOCIATIONS BETWEEN THE CLUSTERED CARDIOMETABOLIC RISK SCORE WITH ENDOTHELIAL FUNCTION, ARTERIAL ELASTICTY AND ARTERIAL STIFFNESS

None of the vascular outcomes were significantly associated with the CMR score at a group level or with the data stratified by sex in either the crude or adjusted models (Table 4.18).

Table 4.18 – Associations of vascular outcomes with cardiometabolic risk score

	Model 1 (crude)		Model 2		Model 3	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
FMD						
Group - CMR	0.057 (-0.142 to 0.256)	0.57	0.266 (-0.004 to 0.536)	0.06	0.244 (-0.016 to 0.504)	0.07
Boys - CMR	0.043 (-0.275 to 0.361)	0.79	0.255 (-0.091 to 0.600)	0.15	0.232 (-0.103 to 0.567)	0.17
Girls - CMR	0.092 (-0.179 to 0.362)	0.50	0.269 (-0.100 to 0.637)	0.15	0.252 (-0.118 to 0.621)	0.18
DC						
Group - CMR	-0.004 (-0.008 to 2.30E-04)	0.06	-0.005 (-0.009 to 1.45E-04)	0.06	-0.005 (-0.010 to -3.69E-05)	0.051
Boys - CMR	-0.004 (-0.010 to 0.001)	0.13	-0.005 (-0.011 to 0.001)	0.10	-0.006 (-0.012 to 0.001)	0.08
Girls - CMR	-0.004 (-0.008 to 0.001)	0.15	-0.004 (-0.010 to 0.001)	0.14	-0.005 (-0.010 to 0.001)	0.13
PWV						
Group - CMR	-0.089 (-0.194 to 0.016)	0.10	-0.074 (-0.180 to 0.033)	0.17	-0.049 (-0.146 to 0.048)	0.32
Boys - CMR	-0.102 (-0.241 to 0.038)	0.15	-0.093 (-0.233 to 0.047)	0.19	-0.070 (-0.202 to 0.061)	0.29
Girls - CMR	-0.084 (-0.197 to 0.029)	0.14	-0.053 (-0.160 to 0.055)	0.33	-0.028 (-0.130 to 0.073)	0.58

FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; CMR = cardiometabolic risk score
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class, baseline vessel diameter (when FMD was the outcome) and the time between cardiometabolic risk assessment and vascular outcome measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index

Chapter 5

Discussion

The aim of this thesis was to examine the associations between PA and ST with endothelial function, arterial elasticity, arterial stiffness and clustered CMR in a large, population sample of children aged 10 y in the UK. This chapter will focus on providing a critical discussion of the results and tie this with the existing literature. It will also discuss the implications of the findings, the strengths and limitations of the study and finish with recommendations for future research.

5.1 SUMMARY OF MAIN FINDINGS

The novelty of this work stems from the use of CoDA, using a homogenous sample in terms of age to investigate the associations, and the inclusion of multiple known confounding variables in the analytical models. The main findings of the present study were that the PA behaviours, specifically LPA, MVPA and ST (relative to the remaining behaviours) were not significantly associated with endothelial function, arterial elasticity, or arterial stiffness overall, or when stratified by sex, after adjustment for confounding variables. These findings did not differ when using CoDA to account for collinearity between the activity behaviours which comprise 100% of the waking day, or while using non-compositional multiple linear regression models. In CoDA models the proportion of time spent in ST (relative to LPA and MVPA) was positively associated with the clustered CMR score at the group level and in the girls, but not in the boys. Additionally, MVPA (relative to LPA and ST) was inversely associated with the clustered CMR score in the group and when analyses were stratified by sex. Using non-compositional multiple linear regression models, the results differed with MVPA inversely associated with the clustered CMR score at a group level only. In the girls, ST was positively, and LPA negatively associated with the clustered CMR score but not for the boys. Finally, the clustered CMR score was

not associated with endothelial function, arterial elasticity, or arterial stiffness. Together, these data suggest, PA, ST and CMR are not associated with vascular health in children aged 10 y. Furthermore, associations between PA and ST with CMR in children are affected by the choice of analytical approach.

5.2 COMPARISONS BETWEEN DATA FOR THE SAMPLE IN THIS THESIS WITH EXISTING COHORT AND NATIONAL DATA

5.2.1 Physical activity and sedentary time

Comparisons of average min spent in PA intensities and ST values between cohort of surveillance studies is challenging due to socioeconomic, environmental, and demographic variation in the samples which can influence time spent performing PA and SBs. There is also inherent variation between different measurement methods (self-report vs. devices) or between different devices that are used to collect the data. Further variation is introduced from differences in the processing of the accelerometer data for how a valid day is defined such as number of hours of wear time are needed and how periods of non-wear time are classified. Researchers also use different approaches for how many weekdays and how many weekend days are required for the data to be considered valid. The inclusion or exclusion of a weekend day can influence average MVPA because of the significant differences in weekday compared to weekend MVPA, with weekend MVPA typically higher than weekday MVPA (Trost et al., 2000). Finally, the choice of cut-points used to classify time spent in different intensity categories introduces additional variation into the accelerometer data. For example, even within the ALSPAC dataset by using the Mattocks et al. (2007) thresholds compared to the Evenson et al. (2008) thresholds on the same participants there are large disparities between the

average min·day^{-1} spent in ST, LPA and MVPA with the two approaches. As presented in Table 3.2, the average time spent in MVPA for the sample in this thesis was 57.6 ± 29.6 min·day^{-1} using the Evenson et al. (2008) thresholds but it was 23.4 ± 15.6 min·day^{-1} using the Mattocks et al. (2007) thresholds.

Average MVPA (min·day^{-1}) reported in the present study using the Evenson et al. (2008) thresholds is in line with data from children aged 9-11 y from the 12 countries (*n*=6,128) participating in the International Study of Childhood Obesity, Lifestyle and the Environment who reported on average spending 60.3 ± 24.9 min·day^{-1} in MPVA (Roman-Vinas et al., 2016). Data from a subsample of the International Children's Accelerometry Database (ICAD) (8 longitudinal studies) reported an average of between 246 and 387 min·day^{-1} in ST for children aged between 5.6 y and 11.7 y at the baseline measurement (van Ekris et al., 2020) which is in line with the data in the present thesis (354.5 ± 72.6 min·day^{-1}). It is important to note that the ICAD does contain ALSPAC data but this is reported using the Mattocks et al. (2007) and Mitchell et al. (2012) thresholds rather than the Evenson et al. (2008) thresholds used in the present thesis. Despite the variation in the reference values introduced from the use of different cut-points and differences in participant characteristics, the sample in this thesis are representative of children in this age range in terms of their PA and ST levels.

Although the average MVPA (min·day^{-1}) reported in this thesis is close to the 60 min·day^{-1} guideline, only 39.7% of the sample in this thesis met the PA guidelines (an average of ≥60 mins of MVPA per day) (UK Chief Medical Officers', 2019). Data from the Active Lives Survey 2018-2019 (Sport England, 2019a) reported

that 46.8% of 5-16 y olds met the PA guidelines which suggests the present sample were on average less active than population data from England, there are methodological differences which likely explain why the present sample appear to be less active. This is because the Active Lives Survey measured PA by parental or child responses to a questionnaire, rather than the device-derived measure used in the present study. Questionnaire responses are known to be subject to reporting bias (Basterfield et al., 2008) and may have resulted in an overestimation of PA compared to device-derived data. For example, Basterfield et al. (2008) found a mean bias of 122 $min \cdot day^{-1}$ for parental-reported MVPA measured by questionnaire compared to device-derived MVPA. Therefore, the activity levels of the sample in the present thesis are broadly comparable to population data in England. However, these statistics are concerning with less than half of the population meeting the PA guidelines so research needs to understand the health consequences of being active compared to being inactive, and to develop strategies to increase PA levels.

5.2.2 Flow mediated dilation

The average values for FMD in the present thesis (8.13 ± 3.39%) are within the range of values presented in the literature for this age group (unadjusted for baseline diameter mean = 8.75%; 95% CI = 8.02-9.47%) (Hopkins et al., 2015). However, the average value in the present thesis is towards the lower end of the 95% CI in the published values and therefore, some participants fall outside the published values. More recently, age and sex-specific percentiles for brachial artery FMD were published and for 10 y old males the 50^{th} percentile was 7.93% and 8.96% for females (Holder et al., 2021). The average FMD was 7.75 ± 3.28% for males and 8.48 ± 3.45% for females in the present thesis. This means the

ALSPAC values are systematically lower than some of the values presented in the literature but some of the variance could be due to differences in the samples, measurement error or differences in technique. For example, the published values in Hopkins et al. (2015) and Holder et al. (2021) were pooled from multiple studies from Universities in different countries so the data was not collected with the explicit purpose of establishing a dataset of normative values and may not represent true normative values for this population. Therefore, the data presented in the present thesis may be a better reflection of “normal” FMD values since they are from a population sample. Also, recruitment criteria for the published datasets means that the samples were asymptomatic from known CVD or metabolic conditions whereas the present sample is a population sample so likely includes individuals who have health conditions such as obesity or hyperlipidaemia, and youth with these conditions have been shown to have impaired FMD compared to healthy controls, with an average FMD of 4-7%, 3-12% and 8-16% respectively (Fernhall and Agiovlasitis, 2008). Therefore, this may have resulted in the lower average FMD reported in the present thesis, although no significant association was observed between the clustered CMR score and FMD in the current data.

Additionally, the measurement of FMD is also known to be highly operator dependent which could explain some of the variation in values between the present sample and the reference data. However, the reliability of the vascular measurements in the present thesis was high, with less than 5% of variance in the vascular measures explained by the operator (Donald et al., 2010). Furthermore, there are methodological differences between the pooled data and the present study in relation to how images were recorded and how peak dilation was identified. In the present study, the end-diastole images were captured every

3 seconds, rather than for every heartbeat whereas all the data from Hopkins et al. (2015) and Holder et al. (2021) was collected via a continuous recording method. This means the true peak dilation may have been missed in the present study for some participants if peak dilation occurred not in line with the 3 second capture, resulting in a lower FMD value (Black et al., 2008).

It is also important to highlight that no attempt was made to standardise conditions for the FMD measurement in terms of the participant being fasted or free from caffeine for the present thesis whereas these conditions were standardised in the published data. However, the variance from not standardising for caffeine and fried food intake was minimal (Donald et al., 2010).

5.2.3 Pulse wave velocity

Reference data for PWV are traditionally published for the carotid-femoral site rather than between the carotid-radial sites which was used in the present study. A study has recently included reference values for the carotid-radial site for 292 ostensibly healthy children from Canada (median age of 13 y old and IQR 7 to 15) and reported a median value of 6.8 $m \cdot s^{-1}$ with an IQR of between 6.2 and 7.5 $m \cdot s^{-1}$ (Torigoe et al., 2020). The mean value in the present study is just outside the IQR at 7.6 $m \cdot s^{-1}$ indicating that the present sample on average had a higher arterial stiffness than the published data. This may reflect the fact that not all the present sample were healthy since it was a population sample and individuals were not excluded if they had medical conditions that may influence arterial stiffness. In contrast, the data from Torigoe et al. (2020) were not from a population sample of children and adolescents, and the participants were excluded if they were obese (BMI z-score ≥ 2), took medication other than

vitamins, were diagnosed with a chronic condition (e.g. diabetes), had a fever or respiratory infection or had a right arm blood pressure > 95th percentile for their age.

5.2.4 Distensibility coefficient

To the author's knowledge, there are no reference data for the brachial artery DC published in the literature so comparisons between the present sample and reference values are not possible at this time. Therefore, it is unknown if the present sample are representative of the wider paediatric population in term of the brachial artery DC.

5.3 ASSOCIATIONS BETWEEN SEDENTARY TIME AND PHYSICAL ACTIVITY WITH ENDOTHELIAL FUNCTION, ARTERIAL ELASTICITY AND ARTERIAL STIFFNESS

5.3.1 Associations between sedentary time with endothelial function, arterial elasticity, and arterial stiffness

A novel finding of the current study is that there were no significant associations between the proportion of time in device-derived ST (relative to MVPA and LPA) and the vascular outcomes in the crude models. This is the first research to use CoDA to examine associations between activity behaviours and multiple vascular outcomes to account for the collinearity between the activity behaviours which comprise 100% of the waking day. The inferences from the CoDA models in this thesis are in line with previous literature using non-compositional multiple linear regression models for both children aged 6 to 11 y old (Nettlefold et al., 2012, Hopkins et al., 2012, Veijalainen et al., 2016, Haapala et al., 2017) and adolescents aged 11 to 16 y old (Melo et al., 2015, Ascenso et al., 2016) of null

associations between ST and vascular outcomes. Existing research has typically focussed on examining the association between ST with one aspect of vascular health such as endothelial function (Hopkins et al., 2012), arterial stiffness (Veijalainen et al., 2016, Haapala et al., 2017), and arterial compliance (Nettlefold et al., 2012) or cIMT (Melo et al., 2015, Ascenso et al., 2016). These measurements are all indicators of vascular health but measure different aspects, therefore, by only including one vascular measure these studies may have missed observing a significant association between ST and a different aspect of vascular health. The present thesis was able to examine associations between ST with endothelial function, arterial stiffness and arterial elasticity to provide a more comprehensive assessment of vascular health. The addition of cIMT to assess vascular structure in the present thesis would have been beneficial, but data were not collected for this measure at age 10 y in the ALSPAC study so it could not be included in the present analysis.

The associations in the present thesis remained non-significant even after adjustment for a range of additional known covariates (in models 2-4) including some important confounders not always used in the existing literature such as mother's social class, lean mass index and family history of hypertension, diabetes, high cholesterol and vascular disease (Charakida et al., 2012, Sletner et al., 2018, Agbaje et al., 2021b). In existing literature analyses are most commonly adjusted for age, sex and an indicator of adiposity (Konigstein et al., 2020), with a few studies adjusting for additional covariates such as cardiorespiratory fitness and CMR (Veijalainen et al., 2016, Haapala et al., 2017). Cardiorespiratory fitness has been shown to be significantly associated with vascular stiffness outcomes in 329, 8-11 y old children (Agbaje et al., 2019). A

moderate correlation (r = 0.25, *p*=0.004) exists between peak oxygen uptake and FMD scaled to the area under the curve of the shear response in 10 y old children (*n*=129) but not the absolute FMD (%) (Hopkins et al., 2009b). Additionally, the clustering of CMR factors has been shown to impair FMD in asymptomatic children (Celermajer et al., 1992). Therefore, the studies that do not include these variables as covariates in the analytical models may be inflating or masking associations between ST and the vascular outcomes from variation in these variables within the samples. This residual confounding variance could have masked a significant association between ST and the vascular outcomes in children but the present thesis was able to adjust for these additional covariates and found no significant associations.

In the present thesis cardiorespiratory fitness was estimated from a sub-maximal exercise test (PWC_{170}) rather than from direct measurement of maximal oxygen uptake from a cardiopulmonary exercise test. This could have introduced error into the association if cardiorespiratory fitness was under or overestimated for some participants. A previous study has reported a moderate correlation between PWC_{170} (W) and absolute peak oxygen uptake (r = 0.49-0.54) in 11-16 y olds (Bland et al., 2012). Future studies should use direct assessment of cardiorespiratory fitness where this is possible to determine a valid measurement of maximal oxygen uptake such as via a ramp and supramaximal test protocol (Sansum et al., 2019).

Veijalainen et al. (2016) measured total and screen-based ST via parental questionnaire. The use of questionnaires introduces bias into the associations because parents are known to under or overestimate time spent in different

sedentary activities compared to accelerometer determined ST (Bringolf-Isler et al., 2012).

It is important to highlight that the device used in the present thesis when worn on the waist is unable to detect posture. SB is specifically defined by the postures of sitting and lying (Sedentary Behaviour Research, 2012), therefore some activity behaviours such as standing and some LPA will likely have been misclassified as ST. This misclassification of behaviour may have influenced the observed associations between ST and the vascular outcomes in both the present study and previous paediatric literature using accelerometers to determine ST (Nettlefold et al., 2012, Hopkins et al., 2012, Melo et al., 2015, Ascenso et al., 2016, Haapala et al., 2017). Standing time (derived from activPAL accelerometers) in children (11-12 y) was inversely associated with adiposity and positively associated with HDL cholesterol (Stockwell et al., 2019). However, no study has investigated the associations between standing and vascular outcomes to understand how the misclassification of standing time as ST is affecting the association between ST and the vascular outcomes in children or adults. Experimental evidence in adults has investigated the effect of standing breaks (10 min in duration per h) on vascular function found standing was unable to prevent the decline in endothelial function in the popliteal artery from 4 h of uninterrupted sitting in overweight/obese middle aged (38 ± 3 y old) participants (Kruse et al., 2018). This may have been because the participants were habitually sedentary so might require a larger standing stimulus to prevent the decline in function. Further experimental research is required in other adult and paediatric populations to understand the acute effect of standing breaks on vascular function. Epidemiological research would benefit from the use of devices that are

able to determine posture such as activPALs to improve the estimates of ST and should investigate the association between standing and vascular outcomes.

A recent systematic review of six observational studies and one interventional study highlighted that the current evidence for the impact of ST on vascular structure and function was of low quality and limited by small sample sizes and a lack of control for both LPA and MVPA, body mass or adiposity and cardiorespiratory fitness in the analytical models (Konigstein et al., 2020). The authors also suggested future research should investigate the patterning of ST such as the frequency and duration of bouts of ST and the breaks in ST which may be important for health. The present thesis has addressed many of these limitations and the findings indicate that in children, device-derived ST is not associated with direct measures of vascular health in cross-sectional observations after control for key covariates such as both LPA and MVPA, and cardiorespiratory fitness. However, it was beyond the scope of the present thesis to investigate the associations of other measures of ST such as the patterning of ST (e.g. frequency of breaks in sitting time or the duration of continuous sitting bouts) with the vascular outcomes to follow on from work by Carson & Janssen (2011), Carson et al. (2014) and Stockwell et al. (2019) who have examined this with indicators of cardiometabolic health as the outcome variables. Additionally, data were not available to examine the associations for different SBs e.g. screen-time and reading, with the vascular outcomes.

5.3.2 Associations between moderate to vigorous physical activity with endothelial function, arterial elasticity, and arterial stiffness

Although the proportion of time spent in MVPA (relative to LPA and ST) was inversely associated with endothelial function and positively associated with arterial stiffness at a group level in the unadjusted models, after controlling for known confounding factors the association was attenuated to null. This is in contrast to Hopkins et al. (2009b) who found a favourable association between VPA (but not MVPA) and brachial artery endothelial function in 10 y old children. However, the authors used an unconventional analysis approach whereby endothelial function was separated into tertiles, and only a limited number of key covariates (DEXA fat mass and cardiorespiratory fitness) were included in the regression models. This significant positive association was only present in the lowest tertile of FMD and not in the group models so was therefore only in a very small sample of children (*n*=43). In addition, residual confounding variance from the absence of key covariates such as lean mass (Sletner et al., 2018) may have resulted in the association between VPA and FMD remaining significant. Lean mass is positively correlated with baseline diameter (Hopkins et al., 2009a) and since FMD is ratio scaled, lean mass may influence FMD% and inflate the association between VPA and FMD. Abbott et al. (2002) also found that brachial artery FMD was positively associated with habitual PA which was calculated as the ratio of total energy expenditure (from doubly labelled water) to resting metabolic rate (from Schofield's equations (Schofield, 1985)) in children aged 6 to 11 y. A multiple regression model was used in this analysis with age, sex, body fat percentage, baseline diameter and ponderal index (a marker of adiposity) selected as potential covariates. The final model included only age and body fat as covariates, likely in part due to the small sample size (45 children), and the

lack of adjustment for other important confounding factors (such as cardiorespiratory fitness (Agbaje et al., 2019)) may explain why the association remained significant. In the crude model in this thesis, there was an inverse association between MVPA and FMD and a positive association between MVPA and PWV in both CoDA and non-compositional models in the group analyses. These significant associations were attenuated to null with the addition of covariates, demonstrating the importance of controlling for confounding factors to prevent inflating the associations between MVPA and vascular outcomes in paediatric populations.

Existing literature examining the relationships between PA and arterial stiffness or compliance in children is mixed with some papers reporting no associations (Reed et al., 2005) like the present study, while others report significant favourable associations (Nettlefold et al., 2012, Veijalainen et al., 2016, Haapala et al., 2017). More recently, Heil and colleagues (2020) also found the favourable relationship between VPA and PWV in 80, 8-11 y old children became non-significant after inclusion of age, sex, BMI-SD and wear time as covariates in the model. In line with the current study, this further highlights the need for inclusion of these key covariates in the models to prevent incorrect conclusions from being drawn. The small samples sizes in the existing literature have hindered the ability to control for a sufficient number of the key covariates without reducing statistical power. Therefore, residual confounding variance from the missing key covariates could have artificially inflated the associations between PA and arterial structure and function in the literature (Nettlefold et al., 2012, Veijalainen et al., 2016, Haapala et al., 2017).

The study by Veijalainen et al. (2016) used a questionnaire to measure MVPA but questionnaires have been shown to inflate estimates of MVPA compared to accelerometer derived data (Basterfield et al., 2008). Consequently this may inflate or mask associations between MVPA and the vascular outcomes. However, other literature using device-derived measures of MVPA has also reported significant associations between MVPA and the vascular outcomes (Nettlefold et al., 2012, Haapala et al., 2017).

5.3.3 Associations between light physical activity with endothelial function, arterial elasticity, and arterial stiffness

In both the CoDA and non-compositional multiple linear regression models presented in this thesis, there were no significant associations between LPA and any of the vascular outcomes. Few studies have examined the association of LPA with the vascular outcomes but all have reported non-significant associations between device-determined LPA with arterial stiffness (Haapala et al., 2017, Heil et al., 2020) and arterial compliance (Nettlefold et al., 2012) which is in line with the findings of the present thesis. As discussed previously, these studies included a limited number of covariates such as age and sex. Therefore, the associations between LPA and the vascular outcomes could have been masked by residual confounding from differences in known confounding variables such as cardiorespiratory fitness and lean mass (Sletner et al., 2018, Agbaje et al., 2019). The present thesis was able to include a number of additional covariates and confirmed an absence of a significant association between LPA and the vascular outcomes.

5.3.4 Does the analysis approach matter?

For the first time, this thesis utilised a novel approach to handle the PA time data (CoDA) to investigate the associations between PA and ST with endothelial function, arterial elasticity and arterial stiffness in a paediatric population. This approach was chosen because it accounts for collinearity between the PA behaviours which comprise 100% of the waking day. Therefore, CoDA allows for all parts of the waking time activity composition (LPA, MVPA and ST) to be included in the analytical model. In contrast, when using a non-compositional multiple linear regression approach, only two of the three activity behaviours can be included in the analytical model along with adjusting for wear time without collinearity issues (Pedisic, 2014). Consequently, the associations from non-compositional multiple linear regression models are limited because systematic differences in the time spent in the activity behaviour that is not adjusted for could bias the results. For example, LPA is often absent in the regression models examining associations between ST or PA and the vascular outcomes (Hopkins et al., 2012, Ried-Larsen et al., 2013, Melo et al., 2015).

The contrasting results observed for PA and the vascular outcomes in the present study compared to existing literature could have been due to the use of CoDA which enabled the analytical models to be adjusted for both ST and LPA. However, when the analysis was performed following a non-compositional multiple linear regression approach, and with the addition of key covariates such as cardiorespiratory fitness (Agbaje et al., 2019) and lean mass index (Sletner et al., 2018) based upon existing literature (Table 3.7), the associations between PA and the vascular outcomes remained non-significant. Consequently, it can be concluded that the absence of statistically significant associations between PA

and the vascular outcomes which is in contrast to some of the existing literature (Abbott et al., 2002, Hopkins et al., 2009b, Nettlefold et al., 2012, Veijalainen et al., 2016, Haapala et al., 2017) is not due to the use of CoDA.

5.3.5 Adolescence may be the time where significant associations show between physical activity and sedentary time with the vascular outcomes

After adjustment for covariates, the time spent in SB, LPA and MVPA has no significant association with vascular outcomes at this age. Further investigation is warranted because although the associations of ST and PA with the vascular outcomes were not significant at age 10 y in the present cross-sectional study, this does not mean that ST and PA in childhood is not predictive of vascular outcomes in adolescence or adulthood.

PA begins to decline from age 7 and through adolescence, although for some boys MVPA stays relatively stable, (Farooq et al., 2018) and ST increases (Janssen et al., 2016). Furthermore, prospective data indicate favourable significant associations between PA (especially VPA) in late adolescence (9-15 y old) and early adulthood (18-36 y old) with vascular outcomes in adulthood (20-36 y old) (van de Laar et al., 2011, Palve et al., 2014). Therefore, it is plausible to suggest that there has not been enough time exposed to atherosclerotic promoting conditions caused by habitual PA and ST by age 10 to show measurable effects on the vasculature this early on in life.

In a cross-sectional observation, there were no significant association reported between device-derived MVPA or VPA with arterial stiffness or cIMT in 336 adolescents aged 15 y old (Ried-Larsen et al., 2013). It has also been reported

that device-derived MVPA or VPA in childhood (*n*=234; aged 8-10 y) was not significantly associated with arterial stiffness or cIMT in a follow up 6 y later during adolescence (Ried-Larsen et al., 2014). However, an unfavourable longitudinal association between time spent watching television at age 12-13 y old and brachio-ankle PWV has been reported in 14-15 y old Japanese adolescent males (Fujiwara et al., 2018). Research has also shown favourable longitudinal associations between self-reported PA and PWV across a 3 y period (age 14 to 17 y) (Chen et al., 2012) and self-reported leisure-time PA with both FMD and aIMT (Pahkala et al., 2011) in adolescents across a 5 y period. Furthermore, a recent study of children (aged 6-9 y at baseline) with a two y follow up period noted an increase in device-determined VPA was associated with better arterial dilation (change in reflection index in response to an exercise bout) and the 2 y change in MVPA was inversely associated with the resting measure stiffness index (Korhonen et al., 2021). Together these data provide some initial evidence that PA and some SBs, and changes in PA are associated with favourable vascular outcomes in adolescents. Therefore, promoting PA during childhood and adolescence should be a target to prevent adverse subclinical atherosclerotic changes in adolescence. However, some of these associations need confirming with device-derived measurements of PA and supporting further with interventional evidence.

The age of the baseline measurement may also be important because leisure time total PA (via questionnaire) in males and females aged 18-24 y was directly associated with carotid artery distensibility, inversely with Young's elastic modulus and indirectly with a stiffness index 21 y later but the favourable associations between PA data from age 9-15 y and the arterial outcomes were

only present in the males and not the females (Palve et al., 2014). However, the use of questionnaires to assess PA may have resulted in error in these associations from the under or over reporting of PA (Basterfield et al., 2008), and these findings need confirming with device-derived measures of PA.

If ST in childhood is associated with increased CVD risk in adulthood, the associations between ST with vascular outcomes in adolescence may be even stronger due to the decline in PA from childhood (age 7 y) to adolescence (age 15 y) from an average MVPA of 75.5 min·day^{-1} to 51.4 min·day^{-1} for boys, and from 63.4 min·day^{-1} and 41.0 min·day^{-1} for girls (Farooq et al., 2018), and the concomitant increase in ST from 346.5 ± 66.6 min·day^{-1} at age 7 y to 535.4 ± 85.4 min·day^{-1} at 15 y (Janssen et al., 2016). High quality prospective studies are required to clarify how the association between the activity behaviours and vascular outcomes develop in childhood and adolescence using device-derived measurements of PA and ST, with control for the key covariates. These studies could also include cross-lagged analyses to tease out the directionality of the associations. If the data indicates significant beneficial associations for MVPA with the vascular outcomes along with negative associations between ST and the vascular outcomes, it would provide evidence to support the development of interventions and changes in policy that promote limiting and breaking up ST, and increasing MVPA in childhood and adolescence for the primary prevention of CVD. In the most recent update to the PA guidelines, children and adolescents are advised to minimise total ST and to break up periods of long duration ST with at least LPA (UK Chief Medical Officers', 2019), but more evidence is required to refine and support this guidance.

5.3.6 The potential influence of upper vs. lower limb measurements of vascular outcomes on the reported associations

Endothelial function can be measured in both the upper and lower limb but FMD in lower limb does not significantly correlate with FMD in upper limb (Thijssen et al., 2011b). Therefore, the absence of a significant association between PA and ST with brachial artery FMD in the current thesis may not mean there is not a significant association between PA and ST with popliteal or femoral artery FMD. If the effects of ST and PA on the vasculature are localised rather than systemic, as is the case with the impact of exercise in adults (Rowley et al., 2011), upper limb FMD may not be affected by ST and PA, hence the absence of a statistically significant association. Humans are bipedal so most activity or movement occurs in the lower limb. Therefore, if the effects of PA and ST on the vasculature are localised, lower limb FMD measurements are required to uncover these associations. However, it is important to highlight that the prognostic value of lower limb FMD is currently unknown.

Acute interventional studies in adults have shown that there are differences between uninterrupted sitting (a type of SB) induced changes in upper and lower limb FMD, with an impairment in endothelial function only observed in the lower limb i.e., the impact is limb specific (Thosar et al., 2014, Restaino et al., 2015). Although this is yet to be examined formally in a paediatric population, research has shown a transient decline in superficial femoral artery endothelial function with 3 h of prolonged sitting in pre-pubertal females (McManus et al., 2015). Furthermore, there is an increase in prevalence of atherosclerotic lesions in the lower limb compared to other commonly measures vessels such as the carotid (Kroger et al., 1999), suggesting the lower limb is more vulnerable to vascular

damage. It is thought that the structure of the superficial femoral artery, with its higher turbulence and chronically lower shear stress observed in adults, might contribute to the higher incidence of atherosclerotic lesions (Wu et al., 2004, Wood et al., 2006). Therefore, it is possible that by assessing FMD in the brachial artery in the present thesis and the existing observational literature rather than the lower limb vessels, the "true" association between ST and endothelial function has been missed. This may also be the same for arterial stiffness as the present thesis measured PWV across the carotid-radial sites rather than using the gold-standard carotid-femoral sites, which means the measure also did not span the lower limb. Future research should examine the relationship between PA and ST with lower limb endothelial function and carotid-femoral PWV in children and adolescents. This research should also include direct comparisons between measurement locations within the same participants to understand if the relationships are limb-specific.

5.4 ASSOCIATIONS BETWEEN SEDENTARY TIME AND PHYSICAL ACTIVITY WITH A CLUSTERED CARDIOMETABOLIC RISK SCORE

5.4.1 Associations between sedentary time with a clustered cardiometabolic risk score

Previous literature has reported that vascular structure and function is normally altered with the presence of traditional CVD risk factors in children and adolescents (Celermajer et al., 1992, Pauciullo et al., 1994, Fernhall and Agiovlasitis, 2008, Riggio et al., 2010). Therefore, the associations between the activity variables with a clustered CMR score were also investigated in the present thesis. In both the girls and group level analyses, there was a significant unfavourable association between increased proportion of ST (relative to LPA

and MVPA) with a higher CMR score but the association was non-significant in the boys. The significant unfavourable association in the group analysis might be being driven by the significant unfavourable association in the girls. However, because there was not a significant sex interaction, this observation needs confirming with additional research. Furthermore, the present study also observed a significant unfavourable association between ST with the clustered CMR in the girls when using a non-compositional multiple linear regression approach, but the association was non-significant at the group level and in the boys. Although there was not a significant sex interaction, the group result in the CoDA model is likely being driven by the girls and could be partially explained by the greater number of girls included in the present study (*n*=2226 vs. *n*=2051, respectively). Therefore, it appears to be inappropriate to pool the data and analyse at a group level due to the difference in the associations between the sexes. However, due to the smaller sample sizes in much of the previous literature (e.g. in Hay et al. (2012) *n*=605 and in Barker et al. (2018) *n*=534), many studies would likely have been underpowered to examine the associations with the data stratified by sex, hence why the data was pooled and sex was included as a covariate. With the data stratified by sex, the significant unfavourable association may only exist in the girls due to differences in other aspects of ST such as how it is accumulated e.g. short vs. long bouts compared to the boys. This richness of data is missed by simply reporting average ST (min·day^{-1}). The difference could also be because the girls were more sedentary than the boys in both absolute terms (360.8 ± 71.2 vs. 347.6 ± 73.6 min·day^{-1}), and as a percentage of the waking day (46.7% vs. 44.5%). Although the difference in absolute terms (min·day^{-1}) was statistically significant, it was small at ~13 min·day^{-1}.

Alternatively, the differences in the associations between sexes could be due to differences in the type of SB that the girls are engaging in compared to the boys during their ST. Sex differences have been reported in the time spent in different SBs in data from 1,513 UK children aged 10 y old with girls spending more time in non-screen SBs (e.g. reading, doing homework or arts and crafts) and boys spending more time in screen-based SBs (e.g. playing videogames and watching television or videos) (Klitsie et al., 2013). The SBs that the boys typically engage in (television viewing and screen time) are the activities that have been consistently associated with increased CVD risk in paediatric populations (Chaput et al., 2013, Grontved et al., 2014, Barker et al., 2018). Additionally, Chaput et al. (2013) and Barker et al. (2018) examined the associations with total ST, and found ST was not significantly associated with increased CVD risk. In contrast, the sedentary activities that the girls typically engage in have been associated with better health outcomes. For example, a favourable association has been reported between the duration of homework and SBP (Gopinath et al., 2012). Additionally, playing a musical instrument was favourably associated with fasting insulin concentration and listening to music was favourably associated with HDL cholesterol and unfavourably associated with TAG in 6-8 y old children (*n*=468) (Vaisto et al., 2014). It is therefore surprising that in the present thesis, the girls had a significant positive association between ST and CMR rather than the boys. Data were not available on the types of activities that the children were engaging in during their ST so it is unknown if this sample of girls simply spent more time watching television and on screens than the boys. Furthermore, the absence of data for time spent in different SBs meant that differences in the

associations between different SBs could not be formally explored as part of the present thesis.

The existing paediatric literature provides some evidence to suggest certain SBs may be more detrimental to health than others. However, with SB being clearly defined as simply an energy expenditure of <1.5 METs while sitting or reclining (Sedentary Behaviour Research, 2012), the reason why individuals are in this activity behaviour should not make a difference. Instead, these differences may exist from variance in the dose from the different SBs (e.g. do people spend more time watching television than they do completing homework?) or due to variance in our ability to measure time spent in these activities accurately. For example, it may be easier for participants to report time spent watching television compared to other activities such as reading because programmes have a set duration. Furthermore, these differences may present due to confounding variance from either co-occurring behaviours such as increased snacking during screen time and television viewing (Pearson and Biddle, 2011, Thivel et al., 2013, Shang et al., 2015) or residual confounding from socioeconomic status or social patterning of certain SBs. More research is needed into SBs other than screen-based activities where the majority of evidence has been focussed on to date, with appropriate control for key confounding variables.

Although Ekelund et al. (2007) also observed a significant unfavourable association between ST (% of wear time) with a CMR score and individual CMR factors, the majority of previous research has reported no significant relationships between ST and individual cardiometabolic risk factors or a clustered CMR score (Ekelund et al., 2012, Hay et al., 2012, Barker et al., 2018). However, this

association has only been examined at a group level with sex added in as a covariate in the models. Therefore, it is possible that the association was been missed by Ekelund et al (2012), Hay et al. (2012) and Barker et al. (2018) due to the boys having no significant relationship between ST and the clustered CMR score. The data from the boys may be driving the group results in the previous studies and consequently resulted in the association being attenuated to null. The group results from the non-compositional multiple linear regression approach in the present thesis were in line with Ekelund et al (2012), Hay et al. (2012) and Barker et al. (2018), providing support for this suggestion of the boys data driving the non-significant association in the existing literature. The difference in results between the non-compositional multiple linear regression approach and CoDA models is likely due to the disparities in the analytical approach and the subsequent inclusion of LPA in the CoDA model. The addition of LPA into the CoDA model allows for the differences in the time spent in LPA between participants to be controlled for and without doing this in the non-compositional multiple linear regression approach, it could have resulted in the masking of the significant positive association between ST and CMR at the group level in the results from Ekelund et al. (2012). Hay et al. (2012) and Barker et al. (2018) used a different non-compositional multiple linear regression approach where all the PA intensities were included as covariates (LPA, MPA and VPA) but wear time was not included. The absence of wear time in analytical models is also inappropriate because differences in wear time can artificially drive differences in the activity behaviours between participants (Tudor-Locke et al., 2011).

Recently, the CoDA approach has been used in a few paediatric papers to examine associations between the ST and individual CMR factors (Carson et al.,

2016b, Carson et al., 2019, Matriccaini et al., 2021). Similar findings to the present thesis have been observed for individual risk factors with ST (relative to the remaining behaviours) found to be unfavourably associated with waist circumference in 6-17 y old children and adolescents (Carson et al., 2016b, Carson et al., 2019). Carson et al. (2016b) also found additional unfavourable associations between ST (relative to the remaining behaviours) and BMI z-score and aerobic fitness (measured via the Modified Canadian Aerobic Fitness Test (Stephens and Craig, 1985, CSEP, 2003)) respectively. Furthermore, Matriccaini et al. (2021) reported an unfavourable association between ST (relative to the remaining behaviours) and a composite metabolic syndrome score in 1,073 12 y old children but the unfavourable associations with BMI and DBP were diminished after a Holm Sequential Bonferroni adjustment was applied, indicating that the unadjusted associations were false positives. Therefore, taken together the present findings in this thesis and current literature indicate ST is associated with poorer cardiometabolic health in youth. Although there was not a significant sex interaction, the findings of this thesis suggests that this may only be present for girls. Therefore, further research is needed with data stratified by sex to confirm the present findings. Additionally, prospective studies are required to understand the causal directions.

5.4.2 Associations between moderate to vigorous physical activity with a clustered cardiometabolic risk score

The results from this thesis showed significant favourable associations between the proportion of time in MVPA (relative to LPA and ST) and the clustered CMR score at both a group level, and when stratified by sex. The group level results support findings from an existing study of 20,871 children and adolescents aged

4-18 y (Ekelund et al., 2012), and highlights the importance of promoting MVPA in childhood and adolescence for CMR benefits. However, the results are in contrast to Bailey et al. (2012) who reported no significant correlation between MVPA (or VPA) and a CMR score in 100 children and adolescents (10-14 y old). This difference in findings between the present thesis and Bailey et al. (2012) may be due to the smaller size and lack of statistical power to detect a significant association, or due to the difference in statistical analysis technique and resulting absence of covariates in the correlation. In another paediatric study (*n*=67) inactive 10-12 y old children (<60 mins MVPA per day) had a significantly higher clustered CMR score than the active children (>60 min MVPA per day) (Boddy et al., 2014). These data along with many other seminal papers (Brage et al., 2004, Andersen et al., 2006, Ekelund et al., 2007, Tarp et al., 2018) form the foundation of the evidence which has led to the current UK PA guidelines which advise children and adolescents to engage in at least 60 min of MVPA on average per day across the week (UK Chief Medical Officer, 2019).

The absence of a difference in the associations between the sexes in the present thesis is in contrast to Rey-Lopez et al. (2013) who found device-derived MVPA was favourably associated with a clustered CMR score in adolescent boys (*n*=376; 13-18 y old) but not in the girls (*n*=393). However, Rey-Lopez et al. (2013) only included age and maternal education level as covariates in the model and therefore systemic differences in other variables such as accelerometer wear time (Tudor-Locke et al., 2011) and time in the other activity behaviours could be masking a significant favourable association between MVPA and the clustered CMR score in the girls.

The majority of the research has examined MVPA as the predictor of CMR (Ekelund et al., 2012, Bailey et al., 2012, Rey-Lopez et al., 2013, Boddy et al., 2014, Ried-Larsen et al., 2014). Few studies have specifically examined MPA and VPA as separate predictors (Hay et al., 2012, Barker et al., 2018, Ekelund et al., 2007). The 60 s epoch for the accelerometer data used in this thesis is not favourable for measuring MPA and VPA as it underestimates time spent in both intensities compared to a 1 s epoch, so MPA and VPA were combined. For example, leisure time VPA was only 3.4 ± 6.2 min·day^{-1} using a 60 s epoch but 11.6 ± 11.2 min·day^{-1} using a 1 s epoch (Sanders et al., 2014). Differences in how the exposure is expressed could explain some of the differences in the results between studies, especially if VPA may be driving the associations between MVPA and CMR. A significant favourable relationship between VPA (but not MVPA) and a clustered CMR score in adolescents, after controlling for sex and biological maturity has been reported (Ried-Larsen et al., 2014), suggesting it may be important to promote VPA specifically in youth. However, this study did not account for time spent in the other activity behaviours in the model, other than television viewing, and only included sex, biological maturation, height, soft-drink consumption and MAP as additional covariates in the model. Therefore, the association between VPA and CMR may be artificially inflated from residual confounding from the influence of the other activity behaviours. Significant favourable associations between MPA and VPA (% of wear time) with a CMR score and individual CMR factors such as SBP, DBP, glucose and insulin concentration were observed by Ekelund et al. (2007) in 9-10 y old and 15-16 y old children and adolescents (*n*=1709). Furthermore, Barker et al. (2018) also observed the significant favourable associations but only when the other activity

behaviours and cardiorespiratory fitness were not included as covariates in the models.

The difference in results of the present thesis compared to Bailey et al. (2012), Rey-Lopez et al. (2013) and Barker et al. (2018) could be due to differences in the calculation of the clustered CMR score. Currently, there is not a standard CMR score to use for paediatric populations in the literature with numerous approaches used (Stavnsbo et al., 2018). Therefore, the inclusion or exclusion of different variables, such as the absence of glucose in the present thesis and inclusion of maximal oxygen uptake by Rey-Lopez et al. (2013), may also be playing a role in the differences in the associations between studies.

The inclusion or exclusion of cardiorespiratory fitness as a covariate in the model also appears key in determining whether the associations between MVPA, MPA or VPA with CMR are significant. The absence of cardiorespiratory fitness from the models by Barker et al. (2018), resulted in significant favourable associations between MPA and VPA with the CMR score, but these associations were attenuated after the inclusion of cardiorespiratory fitness in the model. Furthermore, the present thesis had an indirect measure of cardiorespiratory fitness calculated from a submaximal test (PWC_{170}) in contrast to the 20 m shuttle run test used by Barker et al. (2018) which has been shown to be a better indicator of cardiorespiratory fitness than the PWC_{170} (Mahoney, 1992). The residual confounding variance from a submaximal estimation of cardiorespiratory fitness could therefore, be artificially inflating the associations between MVPA and the CMR score in the present thesis. However, although the 20 m shuttle run is a maximal exercise test, it is still not a direct measurement of cardiorespiratory

fitness so introduces error into the regression models when compared to direct measurement such as via a combined ramp and supramaximal protocol (Sansum et al., 2019).

The present thesis examined associations between the activity behaviours and a clustered CMR score using CoDA models whereas the existing CoDA literature has reported comparable findings between the activity behaviours and individual CMR factors. For example, Carson et al. (2016b) reported favourable associations between MVPA (relative to the remaining behaviours) and BMI z-score, waist circumference, SBP, C-reactive protein concentration and plasma insulin concentration in 4,169 children and adolescents aged 6-17 y old. Additionally, Matriccaini et al. (2021) reported similar favourable associations between MVPA (relative to the remaining behaviours) and BMI, DBP, a composite metabolic syndrome score (sum of the z-scores of MAP, TAG, glucose and waist circumference, minus the z-score of HDL) ApoB/A1 (alternative measure for the ratio between HDL/LDL cholesterol). However, the association with glycoprotein acetyls (a novel inflammatory biomarker) was diminished after a Holm Sequential Bonferroni adjustment was applied, indicating the unadjusted association was a false positive. Less support exists for the benefits of MPA specifically, with Carson et al. (2019) reporting the favourable relationship between MPA (relative to the remaining behaviours) with HDL-cholesterol (adjusted for age, sex, ethnicity, socioeconomic status, total energy intake, sodium and saturated fat) became non-significant after an additional adjustment for waist circumference. In contrast, VPA (relative to the remaining behaviours) was favourably associated with HDL-cholesterol, BMI z-score and waist circumference. These data suggest that VPA may be driving the significant

favourable associations between MVPA and the individual cardiometabolic outcomes and possibly the clustered CMR score in the present thesis. The existing literature and the results of the present thesis support interventions that promote MVPA in childhood, but additional research is required to tease out whether this is because of VPA driving the associations.

5.4.3 Associations between light physical activity with a clustered cardiometabolic risk score

The results in the present study showed no significant associations between LPA (relative to MVPA and ST) with the clustered CMR score. Current literature reports associations between LPA and individual risk factors, with LPA (relative to the other behaviours) unfavourably associated with BMI z-score, waist circumference and SBP in group analyses of 4,169 children and adolescents aged 6-17 y old (Carson et al., 2016b), suggesting increasing LPA at the expense of other behaviours is associated with poorer cardiometabolic health. However, Matricciani et al. (2021) reported the positive association of LPA (relative to the other activity behaviours) with BMI was diminished after a Holm Sequential Bonferroni adjustment in 1,073, 12 y old children. These data suggest that LPA may not be that beneficial for cardiometabolic health outcomes but it is important to highlight that the beta coefficient does not simply represent LPA rather it represents LPA relative to MVPA and ST. Therefore, by increasing the proportion of time spent in LPA, the proportion of time in MVPA and ST are decreased, so the unfavourable associations between LPA and cardiometabolic health outcomes may be reflective of a smaller proportion of MVPA rather than high LPA per se.

In contrast to the CoDA analysis, the present thesis found a significant favourable association between LPA and the clustered CMR score in the girls only by using a non-compositional multiple linear regression approach. This may suggest LPA is beneficial for modifying CMR in girls, but this model does not account for time spent in ST (due to collinearity issues), unlike in the CoDA models where the association was non-significant. Therefore, since the girls were also more sedentary than the boys and ST was positively associated with the CMR score in the non-compositional multiple linear regression model for girls, this finding could be confounded by the absence of ST in the non-compositional multiple linear regression model. However, by using a CoDA model, Carson et al. (2019) found an inverse association between device-determined LPA and DBP, suggesting that some individual CMR factors may benefit from LPA. This may have also been a random finding because the participants had a normal DBP with the median DBP at 59 mmHg and the IQR was 60 to 65 mmHg. To date, much of the literature has focussed on examining the benefits of MVPA in paediatric populations, with fewer studies examining the potential benefits of LPA on health. As a result, there is an abundance of evidence to support the PA guidelines of promoting MVPA in childhood and adolescence but there is a dearth of data for the health benefits of LPA. This highlights the need for further research to understand if interventions that promote LPA are beneficial for cardiometabolic health outcomes. Experimental studies can reveal the magnitude of the cardiometabolic responses to a bout of LPA and breaking up ST with bouts of LPA. For example, experimental evidence in adults has shown interrupting sitting time with walking improves postprandial insulin and glucose compared to seven hours of uninterrupted sitting (Pulsford et al., 2017). Investigating the benefits of LPA may be particularly important for groups where replacing high ST with LPA is most

achievable as it may be more accessible and amenable for individuals to engage in compared to MVPA.

5.5 ASSOCIATIONS BETWEEN A CLUSTERED CARDIOMETABOLIC RISK SCORE WITH ENDOTHELIAL FUNCTION, ARTERIAL ELASTICITY AND ARTERIAL STIFFNESS

The present study found that there was no significant association between the clustered CMR score with endothelial function, arterial elasticity, or arterial stiffness. This novel finding is surprising considering the significant inverse association between the clustering of CVD risk factors and FMD reported previously in children and adolescents aged 8-16 y old (Celermajer et al., 1992). In addition, youth with obesity and hyperlipidaemia have impaired FMD compared to healthy controls (Fernhall and Agiovlasitis, 2008). However, the absence of a significant association in children could have been masked in previous literature from the inclusion of adolescents in the sample, who have been exposed to CMR factors for longer and thus may have measurable vascular changes unlike the children. This highlights the importance of examining the associations in homogenous samples for children and adolescents. In contrast, previous literature has also found CMR factor clustering was not associated with arterial stiffness in 485 adolescents aged, 12-14 y old (Walker et al., 2013) supporting the findings of the present study.

Other data from the ALSPAC sample have reported significant associations between CVD risk factors with the vascular outcomes when the participants were older (17 and 24.5 y for subsequent vascular measures), supporting the suggestion that the changes in the vascular outcomes may not be measurable

until later in adolescence. Dangardt et al. (2019) found fat mass at age 17 y was positively associated with PWV and a high total fat mass index between 9 to 17 y was associated with a greater PWV. Both associations are unfavourable for health. Adolescence appears to be a key time for interventions to promote vascular health because restoring normal fat mass index in adolescence was associated with a normal PWV at age 17 y. Additionally, fat free mass and SBP were positively associated with cIMT at age 17 y but fat mass was negatively associated with cIMT (Chiesa et al., 2019). Furthermore, consistent exposure to high lean mass and SBP over a 15 y period (age 9 y to 24 y) was associated with a 7 y increase in cIMT and carotid-femoral PWV (Agbaje et al., 2021a). These findings also led to the suggestion that some of the changes in vascular outcomes observed in adolescence may be simply physiological changes that occur with normal growth, rather than pathological in nature.

5.6 ASSOCIATIONS BETWEEN THE ACTIVITY BEHAVIOURS OR A CLUSTERED CARDIOMETABOLIC RISK SCORE WITH THE VASCULAR OUTCOMES

When evaluating the paediatric evidence, in combination with the results of the present thesis, it is possible that the research showing no association between ST (Nettlefold et al., 2012, Hopkins et al., 2012, Melo et al., 2015, Veijalainen et al., 2016, Haapala et al., 2017, Ascenso et al., 2016) or total PA (Reed et al., 2005) or MVPA and VPA (Ried-Larsen et al., 2013, Ried-Larsen et al., 2014, Melo et al., 2015) with vascular outcomes (or between the clustered CMR score and vascular outcomes) have studied children who are too young. The studies have included participants with an average age between 6 and 15 y old but only three included adolescents older than 14 y old (Ascenso et al., 2016, Ried-Larsen

et al., 2013, Ried-Larsen et al., 2014). Consequently, these individuals may not been exposed to the atherosclerotic promoting conditions from high ST and/or low MVPA for long enough to result in the vascular changes that have been observed in adults (van de Laar et al., 2011, van de Laar et al., 2014, Palve et al., 2014) as the atherosclerotic changes to the vessel will take time. The favourable association between VPA and arterial stiffness can be largely explained (up to 70%) by simultaneous associations with traditional CVD risk factors (van de Laar et al., 2011), whereas the unfavourable association between television viewing and arterial stiffness can only be partially (20-30%) explained by the associations with traditional CVD risk factors (van de Laar et al., 2014). Therefore, the association between PA and SBs with vascular health is partially mediated by traditional CVD risk factors, although less so for some SBs such as television viewing. Few paediatric studies examining the associations between PA and ST with the vascular outcomes include a clustered CMR score or a comprehensive number of individual CVD risk factors as covariates (Pahkala et al., 2011, Veijalainen et al., 2016, Haapala et al., 2017). In the present thesis, a clustered CMR score was included as covariate in the models examining associations between PA and ST with the vascular outcomes, and the clustered CMR score was not significantly associated with the vascular outcomes. Therefore, the absence of a statistically significant association between PA and ST with the vascular outcomes is likely to be the true observation.

5.7 STRENGTHS AND LIMITATIONS

This study is the first to examine the cross-sectional associations between ST, LPA and MVPA with endothelial function, arterial elasticity, arterial stiffness and a clustered CMR score in a large, population sample of children using CoDA to

address collinearity issues associated with the mutual adjustment for all parts of the activity composition. Previous literature has used a non-compositional multiple linear regression model approach and entered the PA variables in the form of average min·day^{-1}, which fails to take into account the properties of compositional data and therefore, potentially elicited misleading results. By failing to include LPA or ST as covariates in the non-compositional regression models, the variance that they explain could be masking or inflating the observed associations. Furthermore, some of the research using non-compositional multiple linear regressions does not include wear time as a covariate which adds further error into the models because the absence of wear time can artificially drive differences in measured behaviour between participants, especially for ST (Tudor-Locke et al., 2011). This thesis used both CoDA and non-compositional multiple linear regression models and found the same inferences for the vascular outcomes between the two analytical approaches, with none of the activity behaviours being significantly associated with any of the vascular outcomes in the adjusted models. However, for the associations between the activity behaviours and the clustered CMR score there were differences in the findings. In the CoDA models, ST was positively associated with CMR in group and girls data but in non-compositional multiple linear regression models this significant association was only observed in the girls data. Additionally, MVPA was inversely associated with CMR in group data and when data was stratified by sex in the CoDA model but the association was only significant in the group analysis for the non-compositional multiple linear regression models. Furthermore, the non-compositional multiple linear regression model also showed an inverse association between LPA and CMR in girls data that was not observed in the CoDA models. These differences between results were in part likely due to the

differences in the analytical approach. The CoDA models enabled all parts of the composition to be included in the analytical model whereas the non-compositional multiple linear regression models had either LPA or ST removed. Despite the analytical benefit of using CoDA in comparison to a non-compositional multiple linear regression approach, there are limitations from using CoDA in that by only focusing on average time spent in each broad intensity category, differences in other important dimensions of PA are ignored such as volume, frequency, intensity, patterning and duration of PA bouts, which may also be important for health outcomes (Gupta et al., 2020a). In addition, the present thesis examined the cross-sectional associations so the direction of causality was not able to be determined. Therefore, it is currently unknown if these associations are bi-directional or reciprocal. Future studies should examine the longitudinal associations to understand the direction of the associations and whether PA and ST in childhood predicts vascular health in late adolescence and early adulthood, especially if the vascular changes are not observable until adolescence. The ALSPAC dataset could be used to examine these research questions because the participants have been assessed for PA and ST (accelerometer determined) at age 13 y and 15 y, and for PWV and cIMT at age 17 y and 24 y.

This is also the first study examining associations between PA and ST with vascular outcomes to comprehensively control for a number of important confounding variables (such as cardiorespiratory fitness (Agbaje et al., 2019) and lean mass (Sletner et al., 2018)) in the regression models, made possible by our large sample size. However, using multiple covariates could cause other collinearity issues but this was not the case in the present study as the variance inflation factors were <4. Additionally, the large sample size provided the

opportunity to stratify the analysis by sex which is important due to the known sex differences in PA and ST, the vascular outcomes and CMR between boys and girls (Steele et al., 2009, Donald et al., 2010, Stavnsbo et al., 2018). Through this, the sex-dependent associations between ST and the clustered CMR score were discovered, with the significant positive association only present in the girls. At present, there are no differences in the PA and ST guidelines for boys and girls (UK Chief Medical Officer, 2019) but sex-specific guidelines may be warranted if additional research finds sex-dependent associations like the present thesis.

A direct measure of endothelial function via the non-invasive method of flow mediated dilation was performed following standardised and recommended procedures (Thijssen et al., 2019). This method is technologically challenging and provides valuable insight into the health of the artery because changes to vascular function precede changes to vascular structure (Juonala et al., 2004). The clinical significance of a change in FMD in childhood is unknown, but in adults a 1% increase in FMD is associated with a 10-13% reduction in future risk of a CV event (Inaba et al., 2010). Although no assessment of endothelial independent vasodilation was made in the present thesis using a nitric oxide donor such as nitroglycerin, FMD performed in this manner is understood to be endothelium dependent (Green, 2005). The gold standard measure of arterial stiffness is carotid-femoral PWV (Van Bortel et al., 2012) but arterial stiffness can also be measured at the carotid-radial site (Laurent et al., 2006) as was used in the present study. The clinical relevance of peripheral sites is not as well established as for the carotid-femoral site, however, only the carotid-radial site was examined at age 10. Therefore, although the present study does not have

arterial stiffness measures at the gold standard site, it can still provide valuable insight into determinants of arterial stiffness in youth.

A device-derived measurement of PA and ST was determined which is not subject to errors from reporting bias unlike self-report measures (Basterfield et al., 2008), so may provide a better estimate of habitual PA and ST. However, the accelerometer data were collected in 60 s epochs which is known to result in an underestimation of MPA, VPA and MVPA in paediatric populations (Sanders et al., 2014, Banda et al., 2016, Froberg et al., 2017, Aadland et al., 2020). For example, Sanders et al. (2014) reported leisure time MPA was 40.3 ± 11.9 min·day^{-1} using a 1 s epoch but 34.4 ± 14.3 min·day^{-1} using a 60 s epoch, and for VPA it was 11.6 ± 11.2 min·day^{-1} using a 1 s epoch but only 3.4 ± 6.2 min·day^{-1} using a 60 s epoch. Furthermore, leisure time MVPA was 51.9 ± 18.3 min·day^{-1} using a 1 s epoch but only 37.8 ± 16.1 min·day^{-1} using a 60 s epoch. MPA and VPA were combined as MVPA in the composition for analysis, rather than being entered as separate components to reduce the influence of the underestimation of MPA and VPA on the reported associations. Additionally, CoDA cannot have any part of the composition with a zero value, and if the 60 s epoch had resulted in any participants reporting 0 min of VPA, they would have either had to have been removed, or further processing would have been required to deal with the zero values (Rasmussen et al., 2020). The underestimation of VPA may have masked significant associations between VPA with the vascular outcomes or the clustered CMR score. Aadland and colleagues (2020) recently showed how association patterns between PA intensity and metabolic health are skewed towards lower intensities (i.e. MPA rather than VPA) when longer epochs (e.g. 60 s) are used compared to shorter epochs (e.g. 1 s).

Although the application of cut-points to accelerometry data to broadly classify time spent in each of the activity behaviours is widely used in the literature, the choice of cut-points is a controversial issue with a lack of consensus for which cut-points are the most appropriate to be applied to paediatric data (Kim et al., 2012). The intensity cut-points are derived based upon the relationship between movement in different activities and energy expenditure in small samples (n<163) (Mattocks et al., 2007, Evenson et al., 2008, Pulsford et al., 2011). Therefore, if the relationship between movement and energy expenditure is different in the sample you are applying the cut-points to (i.e. if they are more or less fit than the calibration sample), the cut-point will be in the wrong place and lead to misclassification of activity intensity. The use of different cut-points on the same data can have a large influence on the time spent in each of the activity behaviours which has been demonstrated in the literature (Loprinzi et al., 2012, Banda et al., 2016, Froberg et al., 2017) and in Table 3.2. The decision was made to use the Evenson et al. (2008) cut-points that are favoured in the paediatric literature (Trost et al., 2011) rather than ALSPAC specific ones (Mattocks et al., 2008). Misclassification of time spent in a certain activity behaviour can occur if the cut-point is too high or too low to accurately capture all the time spent in that activity. The use of broad cut-points for MVPA also means someone could perform activities from anything between a brisk walk up to an all-out sprint and it would still be classified as time spent in MVPA, but these activities may have different associations with health outcomes.

The present thesis used valid wear time criteria of three or more days which has been shown to provide a good and reliable estimate of habitual PA in children

(Mattocks et al., 2008). Increasing the number of days decreases the sample size available whereas decreasing the number of days decreases the reliability of the PA estimate. It is important to select a wear time criterion that provides a good estimate of habitual PA while maintaining sample size to avoid inflating or masking associations between PA or ST with the vascular outcomes and CMR score. Furthermore, decreasing the sample size by increasing the number of days required may bias the sample if the participants who remain are healthier and more active than the participants who are excluded because they have fewer valid days of data.

As part of the wear time criteria for the accelerometer data in the present thesis, up to 60 minutes of zero-counts were allowed, in line with recommendations (Chinapaw et al., 2014, Aadland et al., 2018). However, the recommendations suggest that periods of interruption within the 60 minutes of zeros should not be allowed (Aadland et al., 2018). The present thesis allowed for up to two minutes of interruptions because the data were processed by researchers at the University of Bristol (Jago et al., 2019). Therefore, this may have influenced the minutes of sedentary time and wear time reported in the present study. For example, Aadland and collegaues (2018) demonstrated that ST decreased when allowing up to 2 minutes of interruptions in the 60 minutes of zero-counts from 508 ± 95 min·day^{-1} to 498 ± 92 min·day^{-1}. Furthermore, wear time decreased from 820 ± 98 min·day^{-1} to 791 ± 100 min·day^{-1} when 2 minutes of interruptions were allowed within the 60 minutes of zero-counts.

It is also important to highlight that the use of a waist-worn accelerometer may have resulted in some behaviour being incorrectly classified as ST. The definition

of SB means that it is dependent upon the posture of the body, whereby the individual has to either be sitting or reclining (Sedentary Behaviour Research, 2012). The waist-worn device is unable to detect posture unlike newer devices available such as activPAL accelerometers, meaning that time spent standing would have been incorrectly classified as ST. The threshold used to define ST was less than 100 CPM in the present study which could have resulted in some very LPA being misclassified as ST. This may have resulted in an overestimate of the time spent ST which could have affected the associations observed in the present study by inflating or masking significant associations. For example, standing time was inversely associated with adiposity and positively associated with HDL cholesterol in 118 children (11-12 y old) (Stockwell et al., 2019) so could have inflated the associations between ST and the CMR score.

The ALSPAC accelerometer data are limited in that ALSPAC only provide the average PA time metric rather than also having alternative metrics such as pattern of activity or the duration of activity and sedentary bouts which have been shown to be important for cardiometabolic health (Carson et al., 2014, Stockwell et al., 2019, Verswijveren et al., 2021). Raw accelerometer files are held centrally, and additional PA variables can be extracted, but at a cost. Due to budgetary constraints of the present project, additional variables were unable to be acquired to provide further insight into these associations.

Sleep data were not available in this study and consequently, a large proportion of time is unaccounted for in the activity composition. The absence of sleep data could be inflating or masking associations between ST or PA with the vascular outcomes and CMR. Existing literature has reported that sleep (relative to ST,

LPA and MVPA) is negatively associated with BMI z-score, waist circumference and SBP in children and adolescents (Carson et al., 2016b). As research moves towards using 24-h recording periods for activity measurements, the relationships should be re-examined while including sleep time in the activity compositions to understand if this influences the strength of the associations.

A limitation of the present study is the time gap between the clustered CMR, vascular outcome, and the PA and ST measurements due to the study not being designed with the present research questions in mind. Therefore, the PA and ST recorded may not reflect the amount the participants were engaging in when the components of the clustered CMR score, and vascular outcomes were measured. This could have led to error in the association observed if the habitual PA and ST changed between these visits. There was on average 1.1 ± 0.3 y between the vascular and accelerometer visits. In a relatively short period of time, habitual PA and ST levels are unlikely to have changed considerably with a pooled analysis reporting that PA levels decreased by 7% per y from the age of 10 y (Dumith et al., 2011). However, between the CMR score and accelerometer measurements the average time was longer (1.9 ± 0.3 y) and during this time the participants moved from primary to secondary school, and PA declines from age 7 y (Farooq et al., 2018). Consequently, these findings may be subject to increased error if this resulted in a change in PA and ST habits in the children. However, PA and SBs have been shown to track between early and mid-childhood with median tracking coefficients reported as 0.36 and 0.52 respectively (Jones et al., 2013). Total PA for UK children has been shown to track between primary and secondary school (r=0.55), with 50% of participant remaining in the same activity tertile (Metcalf et al., 2015). Therefore, although

PA and ST data were not available for the corresponding measurement points at age 9 y and 10 y, the most active and most sedentary children at age 11 y were likely to have been the most active and most sedentary in the previous years.

The sample for the present analysis was restricted to participants who had valid accelerometer data (minimum 500 min recording per day for ≥3 days, and further restricted to an average wear time of 10 h across valid days) and participants were also required to have a complete set of vascular data. It is therefore important to highlight that the sample included in the present analysis were more active (in terms of MVPA) than the total available sample who wore accelerometers age 11 (minimum 500 min wear time), but were similar in terms of ST and LPA. As a result, it appears that predominantly inactive children were excluded from the analysis in this thesis. Consequently, this could have either inflated or diminished the associations observed between MVPA and the vascular outcomes or CMR score, and may mean the results are not reflective of the wider ALSPAC sample. However, despite this difference in MVPA, the samples were similar in terms of the sex composition and in terms of the mother's social class. It is not entirely unexpected that the wider sample had a lower average MVPA ($min \cdot day^{-1}$) than the thesis in this sample because some of the participants did not meet the inclusion criteria for valid wear time in this thesis so were likely to have shorter recording periods and thus recorded less MVPA.

To reduce the influence of body size on the cardiorespiratory fitness data, the data were allometrically scaled. Although the validity of the allometric scaling method for body mass and lean mass was confirmed in the girls and group data, it was not confirmed in the boys. This is likely due to the use of a group scaling

exponent in line with standardised procedures in the literature (Welsman et al., 1996, Loftin et al., 2016). Therefore, lean mass may still be influencing the cardiorespiratory fitness of the boys. However, the r^2 showed the remaining influence of lean mass was very small after the scaling procedure and it explained less than 1% of the variance. Future research should examine whether sex-specific scaling exponents increase the validity of the allometric scaling method for boys. However, this would mean the cardiorespiratory fitness variable would have different units for each sex.

5.8 FUTURE DIRECTIONS

Additional research is required examining prospective associations between the change in ST and PA with vascular outcomes and CMR to strengthen the evidence base in a large, population-based sample of adolescents. Total PA has already begun to decline by age 7 y (Dumith et al., 2011, Farooq et al., 2018) and consequently, ST increases (Janssen et al., 2016) so adolescence is potentially when young people are most at risk of the negative consequences associated with being inactive and highly sedentary. Furthermore, the adolescents will have been exposed more to the atherosclerotic promoting conditions from high ST and low MVPA so differences in vascular outcomes may be quantifiable by this point. It will also be important to understand whether there are any differences in the associations between MPA and VPA with vascular and cardiometabolic health in order to better inform PA guidelines. However, studies need to collect data with a smaller epoch than the 60s epoch used in the present thesis for a more accurate estimation of MPA and VPA. The trajectory of PA behaviours and the association between vascular and cardiometabolic outcomes also needs to be examined to understand what happens if PA decreases and ST increases from childhood to

adolescence. This will help to establish whether being active as a young child has any positive benefit on late adolescent or early adult outcomes, even if the teenage years are relatively sedentary.

Future research should also consider examining ST in more depth, whether that be investigating the patterning of ST such as short vs. long bouts of uninterrupted ST (Johansson et al., 2020, Verswijveren et al., 2021) or stratifying into time spent in specific SBs. Research already subdivides PA variables into different intensities due to the influence that the different intensities have on health outcomes, so it is a reasonable suggestion to explore ST in the same manner. By treating ST as one variable, we might be oversimplifying it and missing out on important associations with cardiovascular and cardiometabolic outcomes that might only be uncovered if we consider the pattern or bout duration rather than just average ST time. Existing evidence provides some initial support for the type of SB being important in paediatric populations with television viewing associated with a higher brachio-ankle PWV (Fujiwara et al., 2018) and increased clustered CMR score (Barker et al., 2018), and screen time positively associated with waist circumference but negatively associated with HDL (Chaput et al., 2013). Additionally, television viewing but not computer usage has been associated with increased CMR in children and adolescents (Carson and Janssen, 2011). This might be because television viewing and screen-based activities are associated with increased caloric consumption (Pearson and Biddle, 2011, Thivel et al., 2013, Shang et al., 2015) through mechanisms such as “mindless eating” (Boulos et al., 2012). Furthermore, television viewing is positively associated with the consumption of energy-dense snacks e.g. confectionery, sweet biscuits/cookies and crisps/salty snacks (Pearson et al., 2014). Engaging in alternative SBs such

as reading a book or completing schoolwork, may be less problematic for health with an inverse association reported between the duration of homework and SBP (Gopinath et al., 2012). However, there are also surprising data where research has shown a positive association between hours spent on homework on weekdays and BMI z-score in Chinese school children (Ren et al., 2017). In theory, the reason why we are sedentary should not matter as the stimulus will be the same unless there are differences in patterning e.g. there may be more interruptions to sitting with schoolwork compared to watching a film. Therefore, differences in the associations between different SBs and health outcomes may simply be due differences in the socioeconomic patterning of different SBs. Although it is possible to adjust for differences in socioeconomic status in regression models through the inclusion of indices such as mother's social class, these indicators can never capture the full impact of socioeconomic status and patterning of behaviour (Braveman et al., 2005). From the existing literature, screen time appears to show consistent adverse associations with health outcomes (Chaput et al., 2013, Grontved et al., 2014, Barker et al., 2018). However, contemporary research is required that reflects the change in the use of screens over recent decades to include more portable screens such as smartphones and tablets (Saunders and Vallance, 2017). The measurement of screen time also needs to consider that adolescents now often use multiple devices simultaneously (Harrington et al., 2021).

Total screen time being a problem may also be an over simplistic viewpoint. It may depend what participants are doing on the screens (watching television vs. schoolwork), the differences in patterning (e.g. number of interruptions) and influenced by the confounding variance from socioeconomic patterning and co-

occurring behaviours. This highlights the complexity of the current issue in creating a policy that promotes a balance between the activity behaviours that provides the greatest benefits to health.

An extension of this work could be to explore the change in the associations with the clustered CMR score e.g. by replacing ST with MVPA, via compositional iso-temporal substitution (Fairclough et al., 2018, Dumuid et al., 2019), However, the results can differ vastly between datasets due to the requirement to use the baseline mean composition of the sample as the starting point (Dumuid et al., 2019). The approach is also limited in that you are unable to predict the outcome of a specific activity composition (Gupta et al., 2020b). Recently, Gupta et al. (2020b) developed a graphical illustration (ternary plot) to try and better address the question of how does changing the composition of time spent in the different activity behaviours influence the outcome?, but the results are derived from the specific source data so cannot be generalised to the wider population.

An alternative option to epidemiological studies is the use of interventional research to try and fill the gap with a combination of acute and long-term “training” or behaviour change studies. In line with the Bradford Hill criteria for causality, experimental evidence, where it is possible to ascertain, can provide the strongest support for causation of the associations observed in epidemiological studies (Hill, 1965). These interventional studies could focus on manipulating screen time and other SBs found to be associated with detrimental outcomes in the epidemiology literature in order to design effective interventions to limit the negative vascular remodelling from these certain SBs. Uninterrupted sitting (3 h) in pre-pubertal girls has shown a transient decline in lower limb endothelial

function (McManus et al., 2015), and there are numerous important questions that need to be examined as a result of these deleterious effects seen after a short bout of sitting in young girls. This includes questions related to the time course that vascular impairment is elicited from uninterrupted sitting i.e. how long do you need to sit for uninterrupted before FMD is impaired, as has already been investigated in the adult literature (Thosar et al., 2014, Thosar et al., 2015, Restaino et al., 2015, Vranish et al., 2018). The potential influence of sex, age, pubertal status, body composition and habitual PA or ST has on the response should also be investigated to understand if guidelines need to be tailored to different groups based upon these characteristics. The decline in function was prevented by breaking up the sitting time each hour with 10 min of moderate intensity cycling (McManus et al., 2015). This poses additional questions surrounding the type, timing, intensity and the amount of PA that is required to prevent the acute decline in vascular function to inform future PA and ST guidelines.

In the same way research has investigated the use of exercise training or PA interventions to see the benefits of PA on cardiometabolic and cardiovascular health outcomes (Kriemler et al., 2011, Knox et al., 2012, Dobbins et al., 2013, Sun et al., 2013, Bond et al., 2015a, Dagger et al., 2018, Pozuelo-Carrascosa et al., 2018, Lakka et al., 2020, Eloranta et al., 2021), a similar methodology could be applied to see if being habitually less sedentary (via increasing PA) has any influence on resting vascular function and the response to a “sitting challenge” known to typically induce a transient decline in vascular function. This provides an extension to examining prospective data in epidemiological models for the potential benefits of replacing ST with MVPA or LPA. However, the interventional

studies have typically examined FMD in the lower limb because adult literature has shown the response to sitting to be limb specific, with the transient impairment only observed in the lower limb vessels (Thosar et al., 2014, Restaino et al., 2015). This may explain the absence of statistically significant associations between high ST and impaired brachial artery FMD in the epidemiological studies. Future research should examine associations between the activity behaviours and lower limb FMD to see if the associations are also limb specific.

Sedentary activities are rooted in everyday life and are not limited to time spent watching television or playing computer games in the evenings. Primary school children spent ~70% of the time in class sitting (Clemes et al., 2016) and this increases to ~75-88% in adolescent populations (Sudholz et al., 2016, Arundell et al., 2019). Adolescents also sit for more than 15 min in duration for at least one bout per lesson (Sudholz et al., 2016). This makes the school environment an opportune place to start by making the school day less sedentary with interventions that encourage the use of PA breaks. However, there are currently no studies that have investigated whether time spent sitting at school is associated with negative health outcomes or not (after adjustment for behaviours out of school). Adult literature indicates that occupational based sitting is associated with better cardiometabolic health after adjustment for total sitting time and confounders such as total leisure-time PA, occupation and education, whereas television viewing and computer usage was associated with increased cardiometabolic risk (Dempsey et al., 2018). This suggests that the context of sitting (occupation/school vs. leisure time sitting) might be important, but is likely only present due to residual confounding from the socioeconomic patterning of health and behaviour, rather than the sitting exposure per se. Although indicators

of socioeconomic status can be included as a covariate in analytical models, the measurement rarely accounts for the full impact of differences in socioeconomic status (Braveman et al., 2005).

In addition, decreasing time spent sitting at school through PA interventions could also have a knock on effect of reducing PA outside of school (Fremeaux et al., 2011). This could be because children and adolescents have been suggested to have an “activitystat” or set point for energy expenditure from PA (Rowland, 1998, Wilkin et al., 2006). Therefore, to maintain energy expenditure, children and adolescents would compensate for the increase in PA during school by increasing ST outside of school (at the expense of PA). Increased ST outside of school may in fact be more detrimental for health with the associated snacking behaviours linked with television and screen time (Pearson and Biddle, 2011, Thivel et al., 2013, Shang et al., 2015).

5.9 IMPLICATIONS

The findings of this thesis provide initial cross-sectional evidence to suggest that the intensity of the PA that children should engage in may need to be of at least a moderate intensity to gain beneficial cardiometabolic effects, providing support for the UK PA guidelines, which advise an average of 60 min of MVPA per day across the week (UK Chief Medical Officer, 2019). Public policy should aim to encourage the increase of MVPA and decrease of ST in childhood for both the direct and indirect changes via traditional CVD risk factors that may subsequently prevent or delay detrimental vascular changes. Although there was not a significant sex interaction, sex-specific PA and ST guidelines may be required if

differences in the associations between ST and CMR for boys and girls are observed in other studies.

5.10 SUMMARY AND CONCLUSION

Although the data showed no associations between any of the activity behaviours with the vascular outcomes, the presence of positive (unfavourable) and inverse (favourable) associations between ST and MVPA (relative to the remaining activity behaviours) with the clustered CMR score respectively, support interventions that promote MVPA and limiting ST in childhood for the benefits associated with CMR. Therefore, it is possible that exposure to elevated CMR in childhood could lead to vascular changes in late adolescence or in adulthood. However, additional prospective studies are required to understand the causal directions. Childhood is the ideal time for intervention for the primary prevention of CVD through initiatives that promote the increase of PA and minimisation of ST. Further research is required to inform the development of improved evidence-based PA and ST guidelines for children and adolescents, focussing in particular on the amount and length of sedentary bouts, and the intensity of PA breaks required.

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Appendix

Table A.1 – Multiple linear regressions testing the sex by activity variable interaction for the vascular outcomes and cardiometabolic risk score using compositional data analysis for accelerometer data

	Model 1 (crude)		Model 2		Model 3		Model 4	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
FMD								
Sex*STilr$_1$	0.444 (-0.138 to 1.025)	0.13	0.472 (-0.096 to 1.041)	0.10	0.463 (-0.105 to 1.031)	0.10	0.454 (-0.114 to 1.022)	0.11
Sex*LPAilr$_1$	-0.286 (-1.202 to 0.629)	0.54	-0.280 (-1.175 to 0.615)	0.53	-0.299 (-1.194 to 0.596)	0.50	-0.260 (-1.155 to 0.635)	0.56
Sex*MVPAilr$_1$	-0.235 (-0.727 to 0.257)	0.35	-0.257 (-0.739 to 0.224)	0.28	-0.245 (-0.727 to 0.236)	0.31	-0.250 (-0.732 to 0.232)	0.30
DC								
Sex*STilr$_1$	0.002 (-0.009 to 0.012)	0.78	0.002 (-0.009 to 0.012)	0.73	0.002 (-0.009 to 0.013)	0.72	0.002 (-0.008 to 0.013)	0.66
Sex*LPAilr$_1$	0.003 (-0.013 to 0.020)	0.70	0.004 (-0.013 to 0.021)	0.64	0.004 (-0.013 to 0.021)	0.64	0.003 (-0.013 to 0.020)	0.69
Sex*MVPAilr$_1$	-0.002 (-0.0111 to 0.007)	0.65	-0.002 (-0.011 to 0.007)	0.59	-0.003 (-0.011 to 0.006)	0.58	-0.003 (-0.012 to 0.006)	0.56
PWV								
Sex*STilr$_1$	0.059 (-0.151 to 0.270)	0.58	0.043 (-0.171 to 0.257)	0.69	0.044 (-0.170 to 0.257)	0.68	0.046 (-0.168 to 0.259)	0.67
Sex*LPAilr$_1$	0.314 (-0.017 to 0.645)	0.06	0.270 (-0.067 to 0.608)	0.11	0.275 (-0.061 to 0.611)	0.10	0.266 (-0.070 to 0.602)	0.11
Sex*MVPAilr$_1$	-0.133 (-0.311 to 0.045)	0.14	-0.109 (-0.290 to 0.073)	0.23	-0.111 (-0.292 to 0.070)	0.22	-0.109 (-0.290 to 0.071)	0.23
CMR								
Sex*STilr$_1$	0.053 (-0.090 to 0.196)	0.46	0.063 (-0.080 to 0.206)	0.38	0.061 (-0.082 to 0.203)	0.39	0.063 (-0.079 to 0.205)	0.38
Sex*LPAilr$_1$	-0.156 (-0.381 to 0.070)	0.17	-0.114 (-0.350 to 0.122)	0.33	-0.121 (-0.355 to 0.114)	0.30	-0.117 (-0.351 to 0.116)	0.32
Sex*MVPAilr$_1$	0.007 (-0.115 to 0.128)	0.91	-0.012 (-0.139 to 0.114)	0.85	-0.009 (-0.135 to 0.118)	0.89	-0.011 (-0.137 to 0.115)	0.86

FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; CMR = cardiometabolic risk

Activity behaviour variables are mean centred

Model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex, age in years from peak height velocity at 10 year clinic (y; or 9 y when CMR was outcome), mother's social class (I to V), baseline vessel diameter (mm; only when FMD was the outcome) and either the time between vascular and accelerometer measurements (y) or time between CMR score and accelerometer measurements (y)

Model three was an extension of model 2 with further adjustment for cardiorespiratory fitness scaled to lean body mass ($W \cdot kg^{0.59}$) and the lean mass index ($kg \cdot m^{-2}$)

Model four was an extension of model 3, with further adjustment for CMR score (apart from when CMR score was the outcome), and family history of hypertension, diabetes, high cholesterol, and vascular disease

Table A.2 – Multiple linear regressions testing the sex by activity variable interaction for the vascular outcomes and cardiometabolic risk score using a non-compositional multiple linear regression approach

	Model 1 (crude)		**Model 2**		**Model 3**		**Model 4**		**Model 5**	
	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value	b (95% CI)	*P*-value
FMD										
Sex*ST	0.002 (-0.003 to 0.001)	0.21	0.002 (-0.001 to 0.005)	0.16	0.002 (-0.001 to 0.005)	0.15	0.002 (-0.001 to 0.005)	0.17	0.002 (-0.001 to 0.005)	0.18
Sex*LPA	-0.003 (-0.006 to 0.001)	0.13	-0.003 (-0.006 to 2.55E-04)	0.06	-0.003 (-0.006 to 2.64-E04)	0.07	-0.003 (-0.006 to 4.22E-04)	0.08	-0.003 (-0.006 to 3.97E-04)	0.08
Sex*MVPA	**-0.010 (-0.007 to -0.002)**	**0.013**	-0.007 (-0.014 to 4.77E-04)	0.06	-0.007 (-0.014 to 0.001)	0.07	-0.007 (-0.014 to 0.001)	0.07	-0.007 (-0.014 to 0.001)	0.07
DC										
Sex*ST	4.18E-06 (-4.59E-05 to 5.43E-05)	0.87	3.35E-06 (-4.78E-05 to 5.45E-05)	0.90	5.47E-06 (-4.59E-05 to 5.68E-05)	0.83	8.84E-06 (-4.26E-05 to 6.02E-05)	0.73	1.00E-05 (-4.14E-05 to 6.14E-05)	0.70
Sex*LPA	-3.22E-06 (-6.42E-05 to 5.77E-05)	0.92	-2.28E-06 (-6.45E-05 to 5.99E-05)	0.94	-2.47E-06 (-6.47E-05 to 5.98E-05)	0.94	-4.57E-06 (-6.68E-05 to 5.76E-05)	0.88	-4.52E-06 -6.68E-05 to 5.77E-05)	0.88
Sex*MVPA	5.16E-05 (-8.44E-05 to 1.88E-04)	0.46	4.16E-05 (-9.78E-05 to 1.81E-04)	0.55	3.32E-05 (-1.07E-04 to 1.73E-04)	0.64	2.84E-05 (-1.11E-04 to 1.68E-04)	0.68	2.67E-05 (-1.13E-04 to 1.67E-04)	0.70
PWV										
Sex*ST	-1.75E-04 (-0.001 to 0.001)	0.74	-2.00E-04 (-0.001 to 0.001)	0.70	-2.65E-04 (-0.001 to 0.001)	0.61	-2.76E-04 (-0.001 to 0.001)	0.59	-2.89E-04 (-0.001 to 0.001)	0.58
Sex*LPA	3.93E-04 (-0.001 to 0.002)	0.53	3.55E-04 (-0.001 to 0.002)	0.57	3.59E-04 (-0.001 to 0.002)	0.57	2.90E-04 (-0.001 to 0.002)	0.64	2.81E-04 (-0.001 to 0.002)	0.65
Sex*MVPA	-0.001 -0.004 to 0.002)	0.57	-0.001 (-0.004 to 0.002)	0.44	-0.001 (-0.004 to 0.002)	0.50	-0.001 (-0.004 to 0.002)	0.50	-0.001 (-0.004 to 0.002)	0.50
CMR										
Sex*ST	3.46E-04 (-3.56E-04 to 0.001)	0.33	3.66E-04 (-3.28E-04 to 0.001)	0.29	4.43E-04 (-2.50E-04 to 0.001)	0.20	4.75E-04 (-2.10E-04 to 0.001)	0.17	4.56E-04 (-2.26E-04 to 0.001)	0.18
Sex*LPA	-0.001 (-0.001 to 2.64E-04)	0.17	-0.001 (-0.001 to 2.94E-04)	0.20	-0.001 (-0.001 to 2.82E-04)	0.19	-0.001 (-0.001 to 3.08E-04)	0.21	-0.001 (-0.001 to 2.96E-04)	0.21
Sex*MVPA	-5.00E-04 (-0.003 to 0.002)	0.66	-0.001 (-0.003 to 0.001)	0.38	-0.001 (-0.003 to 0.001)	0.45	-0.001 (-0.003 to 0.001)	0.43	-0.001 (-0.003 to 0.001)	0.42

FMD = flow mediated dilation; DC = distensibility coefficient; PWV = pulse wave velocity; CMR = cardiometabolic risk

Activity behaviour variables are mean centred

Model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex, age in y from peak height velocity at 10 year clinic (y; 9 y when CMR was outcome), the time between vascular and accelerometer measurement (y) or the time between CMR score and accelerometer measurement (y), and the accelerometer wear time (min·day^{-1})

Model three was an extension of model two with further adjustment for an additional activity variable (min·day^{-1}; MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass (W·kg$^{0.585}$)

Model four was an extension of model three with further adjustment for lean mass index (kg·m^{-2}) and the clustered CMR score (apart from when CMR score was the outcome)

Model five was an extension of model four with further adjustment for family history of hypertension, diabetes, high cholesterol and vascular disease, and mother's social class (I to V)

Table A.3 – Multiple linear regressions testing the sex by activity variable interaction for the vascular outcomes and cardiometabolic risk score using compositional data analysis for accelerometer data (non-imputed data)

	Model 1 (crude)			Model 2			Model 3			Model 4		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
FMD												
Sex*STilr$_1$	0.444 (-0.138 to 1.025)	0.13	4277	0.720 (-0.211 to 1.651)	0.13	1626	0.717 (-0.751 to 2.184)	0.34	779	0.126 (-1.971 to 2.222)	0.91	425
Sex*LPAilr$_1$	-0.286 (-1.202 to 0.629)	0.54	4277	0.019 (-1.401 to 1.439)	0.98	1626	0.562 (-1.601 to 2.726)	0.61	779	0.785 (-2.520 to 4.090)	0.64	425
Sex*MVPAilr$_1$	-0.235 (-0.727 to 0.257)	0.35	4277	-0.504 (-1.278 to 0.270)	0.20	1626	-0.689 (-1.921 to 0.544)	0.27	779	-0.326 (2.129 to 1.476)	0.72	425
DC												
Sex*STilr$_1$	0.002 (-0.009 to 0.012)	0.78	4277	0.008 (-0.009 to 0.025)	0.34	1626	0.012 -0.014 to 0.038)	0.37	779	0.008 (-0.026 to 0.042)	0.65	425
Sex*LPAilr$_1$	0.003 (-0.013 to 0.020)	0.70	4277	-0.007 (-0.033 to 0.019)	0.58	1626	-0.026 (-0.065 to 0.012)	0.18	779	-0.027 (-0.081 to 0.027)	0.32	425
Sex*MVPAilr$_1$	-0.002 (-0.0111 to 0.007)	0.65	4277	-0.004 (-0.018 to 0.010)	0.62	1626	7.71E-06 (-0.022 to 0.022)	>0.99	779	0.002 (-0.027 to 0.031)	0.88	425
PWV												
Sex*STilr$_1$	0.059 (-0.151 to 0.270)	0.58	4277	0.077 (-0.271 to 0.425)	0.66	1626	0.200 (-0.334 to 0.734)	0.46	779	-0.105 (-0.786 to 0.576)	0.76	425
Sex*LPAilr$_1$	0.314 (-0.017 to 0.645)	0.06	4277	-0.040 (-0.571 to 0.491)	0.88	1626	0.107 (-0.680 to 0.895)	0.79	779	-0.014 (-1.090 to 1.061)	0.98	425
Sex*MVPAilr$_1$	-0.133 (-0.311 to 0.045)	0.14	4277	-0.042 (-0.331 to 0.248)	0.78	1626	-0.176 (-0.625 to 0.273)	0.44	779	0.082 (-0.504 to 0.667)	0.78	425
CMR												
Sex*STilr$_1$	0.061 (-0.074 to 0.196)	0.38	2546	0.068 (-0.144 to 0.279)	0.53	1037	-0.101 (-0.410 to 0.209)	0.52	521	-0.062 (-0.415 to 0.292)	0.73	425
Sex*LPAilr$_1$	-0.133 (-0.347 to 0.079)	0.22	2546	0.119 (-0.212 to 0.451)	0.48	1037	0.164 (-0.310 to 0.640)	0.50	521	0.146 (-0.413 to 0.706)	0.61	425
Sex*MVPAilr$_1$	-0.005 (-0.119 to 0.109)	0.93	2546	-0.080 (-0.256 to 0.096)	0.37	1037	0.022 (-0.243 to 0.287)	0.87	521	0.002 (-0.301 to 0.306)	0.99	425

FMD = flow mediated dilation; DC = distensibility coefficient; PWV = pulse wave velocity; CMR = cardiometabolic risk

Activity behaviour variables are mean centred

Model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex, age in years from peak height velocity at 10 year clinic (y; or 9 y when CMR was outcome), mother's social class (I to V), baseline vessel diameter (mm; only when FMD was the outcome) and either the time between vascular and accelerometer measurements (y) or time between CMR score and accelerometer measurements (y)

Model three was an extension of model 2 with further adjustment for cardiorespiratory fitness scaled to lean body mass ($W \cdot kg^{0.59}$) and the lean mass index ($kg \cdot m^{-2}$)

Model four was an extension of model 3, with further adjustment for CMR score (apart from when CMR score was the outcome), and family history of hypertension, diabetes, high cholesterol, and vascular disease

Table A.4 – Multiple linear regressions testing the sex by activity variable interaction for the vascular outcomes and cardiometabolic risk score using a non-compositional multiple linear regression approach (non-imputed data)

	Model 1 (crude)			**Model 2**			**Model 3**			**Model 4**			**Model 5**		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
FMD															
Sex*ST	0.002 (-0.003 to 0.001)	0.21	4277	0.001 (-0.002 to 0.004)	0.43	3449	-0.002 (-0.007 to 0.002)	0.33	1673	-0.002 (-0.008 to 0.003)	0.45	1116	-7.34E-05 (-0.010 to 0.010)	0.99	425
Sex*LPA	-0.003 (-0.006 to 0.001)	0.13	4277	-0.002 (-0.006 to 0.002)	0.27	3449	0.004 (-0.002 to 0.009)	0.18	1673	0.002 (-0.004 to 0.009)	0.52	1116	2.85E-04 (-0.012 to 0.011)	0.96	425
Sex*MVPA	**-0.010 (-0.007 to -0.002)**	**0.013**	4277	-0.007 (-0.015 to 0.001)	0.10	3449	-0.010 (-0.022 to 0.002)	0.09	1673	-0.013 (-0.027 to 0.001)	0.07	1116	-0.014 (-0.040 to 0.012)	0.29	425
DC															
Sex*ST	4.18E-06 (-4.59E-05 to 5.43E-05)	0.87	4277	5.16E-06 (-5.18E-05 to 6.21E-05)	0.86	3449	-2.63E-05 (-1.14E-04 to 6.19E-05)	0.56	1673	-4.14E-05 (-1.47E-04 to 6.37E-05)	0.44	1116	4.06E-05 (-1.24E-04 to 2.05E-04)	0.63	425
Sex*LPA	-3.22E-06 (-6.42E-05 to 5.77E-05)	0.92	4277	-1.11E-05 (-8.00E-05 to 5.78E-05)	0.75	3449	1.69E-05 (-1.20E-04 to 8.65E-05)	0.75	1673	-2.20E-05 (-1.44E-04 to 9.98E-05)	0.72	1116	-1.09E-04 (2.95E-04 to 7.65E-05)	0.25	425
Sex*MVPA	5.16E-05 (-8.44E-05 to 1.88E-04)	0.46	4277	4.48E-06 (-1.49E-04 to 1.58E-04)	0.95	3449	-6.37E-05 (-2.97E-04 to 1.69E-04)	0.59	1673	-1.44E-04 (-4.04E-04 to 1.15E-04)	0.27	1116	4.68E-05 (-3.72E-04 to 4.66E-04)	0.83	425
PWV															
Sex*ST	-1.75E-04 (-0.001 to 0.001)	0.74	4277	-0.001 (-0.002 to 3.52E-04)	0.18	3449	-0.001 (-0.002 to 0.001)	0.36	1673	-0.001 (-0.003 to 0.001)	0.24	1116	1.04E-04 (-0.003 to 0.003)	0.95	425
Sex*LPA	3.93E-04 (-0.001 to 0.002)	0.53	4277	3.10E-04 (-0.001 to 0.002)	0.66	3449	-3.65E-04 (-0.002 to 0.002)	0.72	1673	4.44E-04 (-0.002 to 0.003)	0.73	1116	0.001 (-0.003 to 0.004)	0.74	425
Sex*MVPA	-0.001 -0.004 to 0.002)	0.57	4277	-0.001 (-0.004 to 0.002)	0.51	3449	-0.002 (-0.006 to 0.003)	0.48	1673	-0.001 (-0.006 to 0.005)	0.82	1116	0.003 (-0.005 to 0.011)	0.49	425
CMR															
Sex*ST	4.18E-04 (-2.40E-04 to 0.001)	0.21	2546	2.45E-04 (-4.47E-04 to 0.001)	0.49	2152	5.38E-04 (-4.49E-04 to 0.002)	0.29	1116	4.70E-04 (-4.86E-04 to 0.001)	0.33	1116	2.08E-04 (-0.002 to 0.002)	0.81	425
Sex*LPA	-4.38E-04 (-0.001 to 3.56E-04)	0.28	2546	-8.94E-05 (-0.001 to 0.001)	0.83	2152	-1.40E-04 (-0.001 to 0.001)	0.74	1116	2.95E-04 (-0.001 to 0.001)	0.60	1116	0.001 (-0.001 to 0.003)	0.16	425
Sex*MVPA	-2.58E-04 (-0.002 to 0.001)	0.77	2546	-0.001 (-0.003 to 0.001)	0.22	2152	-6.88E-04 (-0.003 to 0.002)	0.58	1116	-4.68E-04 (-0.003 to 0.002)	0.70	1116	0.001 (-0.004 to 0.005)	0.75	425

FMD = flow mediated dilation; DC = distensibility coefficient; PWV = pulse wave velocity; CMR = cardiometabolic risk

Activity behaviour variables are mean centred

Model two was adjusted for age at 10 y clinic (y; or 9 y when CMR was outcome), sex, age in y from peak height velocity at 10 year clinic (y; 9 y when CMR was outcome), the time between vascular and accelerometer measurement (y) or the time between CMR score and accelerometer measurement (y), and the accelerometer wear time (min·day^{-1})

Model three was an extension of model two with further adjustment for an additional activity variable (min·day^{-1}; MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass (W·kg$^{0.585}$)

Model four was an extension of model three with further adjustment for lean mass index (kg·m^{-2}) and the clustered CMR score (apart from when CMR score was the outcome)

Model five was an extension of model four with further adjustment for family history of hypertension, diabetes, high cholesterol and vascular disease, and mother's social class (I to V)

Table A.5 – Associations between physical activity or sedentary time and endothelial function (observed data only) using compositional data analysis for accelerometer data

	Model 1 (unadjusted)			Model 2			Model 3			Model 4		
	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*
Group												
ST : LPA & MVPA	0.038 (-0.331 to 0.407)	0.84	4277	0.057 (-0.542 to 0.656)	0.85	1626	0.224 (-0.689 to 1.367)	0.63	779	-0.337 (-1.677 to 1.004)	0.62	425
LPA : MVPA & ST	0.348 (-0.124 to 0.820)	0.15	4277	0.128 (-0.627 to 0.883)	0.74	1626	0.290 (-0.817 to 1.397)	0.61	779	0.967 (-0.707 to 2.640)	0.26	425
MVPA : ST & LPA	**-0.386 (-0.640 to -0.132)**	**0.003**	4277	-0.185 (-0.606 to 0.236)	0.39	1626	-0.514 (-1.157 to 0.129)	0.12	779	-0.630 (-1.579 to 0.319)	0.19	425
Boys												
ST : LPA & MVPA	-0.286 (-0.796 to 0.223)	0.27	2051	-0.265 (-1.090 to 0.561	0.53	759	0.022 (-1.284 to 1.327)	0.97	318	0.239 (-1.693 to 2.172)	0.81	169
LPA : MVPA & ST	0.304 (-0.362 to 0.970)	0.37	2051	0.242 (-0.828 to 1.311)	0.66	759	0.010 (-1.681 to 1.701)	0.99	318	0.182 (-2.419 to 2.782)	0.89	169
MVPA : ST & LPA	-0.017 (-0.393 to 0.358)	0.93	2051	0.023 (-0.601 to 0.648)	0.94	759	-0.031 (-1.112 to 1.050)	0.95	318	-0.421 (-2.085 to 1.243)	0.62	169
Girls												
ST : LPA & MVPA	0.290 (-0.239 to 0.818)	0.28	2226	0.369 (-0.501 to 1.239)	0.41	867	0.476 (-0.817 to 1.769)	0.47	461	-0.442 (-2.340 to 1.456)	0.65	256
LPA : MVPA & ST	-0.157 (-0.840 to 0.525)	0.65	2226	-0.018 (-1.092 to 1.055)	0.97	867	0.294 (-1.227 to 1.814)	0.70	461	1.135 (-1.164 to 3.434)	0.33	256
MVPA : ST & LPA	-0.132 (-0.521 to 0.256)	0.50	2226	-0.351 (-0.929 to 0.226)	0.23	867	-0.770 (-1.599 to 0.060)	0.07	461	-0.693 (-1.904 to 0.519)	0.26	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class, baseline vessel diameter and the time between vascular and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

Table A.6 – Associations between physical activity or sedentary time and endothelial function (observed data) using a non-compositional multiple linear regression approach

	Model 1 (unadjusted)			Model 2			Model 3			Model 4			Model 5		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
Group															
ST	1.78E-05 (-0.001 to 0.001)	0.98	4277	0.001 (-0.001 to 0.002)	0.52	3449	0.001 (-0.002 to 0.003)	0.68	1673	-0.001 (-0.004 to 0.003)	0.72	1116	-0.004 (-0.010 to 0.003)	0.28	425
LPA	-0.001 (-0.003 to 0.001)	0.30	4277	-0.001 (-0.003 to 0.001)	0.50	3449	-0.001 (-0.003 to 0.002)	0.68	1673	0.001 (-0.003 to 0.004)	0.72	1116	0.004 (-0.003 to 0.010)	0.28	425
MVPA	**-0.006 (-0.009 to -0.003)**	**0.001**	4277	-2.62E-04 (-0.004 to 0.004)	0.90	3449	-0.003 (-0.010 to 0.004)	0.34	1673	-0.004 (-0.013 to 0.004)	0.31	1116	-0.014 (-0.029 to 4.04E-04)	0.06	425
Boys															
ST	-0.001 (-0.003 to 0.001)	0.16	2051	-3.01E-04 (-0.003 to 0.002)	0.81	1609	0.004 (-3.80E-04 to 0.008)	0.07	676	0.003 (-0.003 to 0.008)	0.32	458	4.89E-04 (-0.009 to 0.010)	0.92	169
LPA	0.001 (-0.002 to 0.003)	0.64	2051	-3.24E-05 (-0.003 to 0.003)	0.98	1609	-0.004 (-0.008 to 3.78E-04)	0.07	676	-0.003 (-0.008 to 0.003)	0.32	458	-4.97E-04 (-0.010 to 0.009)	0.92	169
MVPA	0.001 (-0.004 to 0.005)	0.79	2051	0.001 (-0.004 to 0.007)	0.57	1609	0.006 (-0.004 to 0.017)	0.24	676	0.005 (-0.007 to 0.018)	0.40	458	-0.001 (-0.027 to 0.025)	0.92	169
Girls															
ST	4.73E-04 (-0.002 to 0.002)	0.65	2226	0.001 (-0.001 to 0.004)	0.25	1840	-0.002 (-0.006 to 0.002)	0.35	997	-0.003 (-0.007 to -0.002)	0.25	658	-0.004 (-0.013 to 0.005)	0.37	256
LPA	-0.002 (-0.004 to 3.44E-04)	0.09	2226	-0.001 (-0.004 to 0.002)	0.39	1840	0.002 (-0.002 to 0006)	0.35	997	0.003 (-0.002 to 0.007)	0.25	658	0.004 (-0.005 to 0.013)	0.37	256
MVPA	**-0.007 (-0.013 to -0.001)**	**0.032**	2226	-0.004 (-0.011 to 0.003)	0.27	1840	**-0.011 (-0.021 to -0.002**	**0.023**	997	**-0.012 (-0.023 to -0.001)**	**0.037**	658	-0.019 (-0.037 to 1.64E-04)	0.05	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, baseline vessel diameter, the time between vascular and accelerometer measurement and accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

Table A.7 – Associations between physical activity or sedentary time and arterial elasticity (observed data only) using compositional data analysis for accelerometer data

	Model 1 (unadjusted)			Model 2			Model 3			Model 4		
	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*
Group												
ST : LPA & MVPA	-0.003 (-0.010 to 0.004)	0.36	4277	-0.002 (-0.013 to 0.009)	0.74	1626	-0.003 (-0.019 to 0.013)	0.70	779	-0.006 (-0.028 to 0.015)	0.56	425
LPA : MVPA & ST	0.003 (-0.006 to 0.113)	0.50	4277	0.003 (-0.010 to 0.017)	0.63	1626	0.006 (-0.012 to 0.027)	0.46	779	0.017 (-0.011 to 0.044)	0.23	425
MVPA : ST & LPA	1.70E-03 (-0.004 to 0.005)	0.94	4277	-0.002 (-0.009 to 0.006)	0.70	1626	-0.004 (-0.016 to 0.007)	0.48	779	-0.010 (-0.025 to 0.005)	0.20	425
Boys												
ST : LPA & MVPA	-0.003 (-0.013 to 0.007)	0.55	2051	-0.007 (-0.022 to 0.007)	0.33	759	-0.015 (-0.039 to 0.009)	0.21	318	-0.017 (-0.049 to 0.015)	0.29	169
LPA : MVPA & ST	0.003 (-0.010 to 0.015)	0.68	2051	0.009 (-0.010 to 0.029)	0.35	759	0.026 (-0.005 to 0.057)	0.10	318	0.038 (-0.005 to 0.081)	0.08	169
MVPA : ST & LPA	-6.79E-03 (-0.007 to 0.007)	0.94	2051	-0.002 (-0.013 to 0.009)	0.75	759	-0.011 (-0.031 to 0.009)	0.28	318	-0.021 (-0.048 to 0.007)	0.13	169
Girls												
ST : LPA & MVPA	-0.003 (-0.012 to 0.006)	0.52	2226	0.004 (-0.012 to 0.020)	0.64	867	0.006 (-0.016 to 0.029)	0.57	461	-0.002 (-0.032 to 0.029)	0.92	256
LPA : MVPA & ST	0.005 (-0.007 to 0.017)	0.40	2226	-0.002 (-0.021 to 0.018)	0.86	867	-0.004 (-0.030 to 0.023)	0.79	461	0.007 (-0.029 to 0.044)	0.70	256
MVPA : ST & LPA	-0.002 (-0.009 to 0.005)	0.56	2226	-0.002 (0.013 to 0.009)	0.71	867	-0.003 (-0.017 to 0.011)	0.69	461	-0.006 (-0.025 to 0.014)	0.57	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class and the time between vascular and accelerometer measurement

Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index

Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

Table A.8 – Associations between physical activity or sedentary time and arterial elasticity (observed data) using a non-compositional multiple linear regression approach

	Model 1 (unadjusted)			Model 2			Model 3			Model 4			Model 5		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
Group															
ST	-6.35E-06 (-3.13E-05 to 1.86E-05)	0.62	4277	-7.83E-06 (-3.98E-05 to 2.42E-05)	0.63	3449	-3.51E-05 (-9.07E-05 to 2.06E-05)	0.22	1673	-3.32E-05 (-9.92E-05 to 3.27E-05)	0.32	1116	-5.56E-05 (-1.61E-04 to 4.99E-05)	0.30	425
LPA	2.32E-05 (-7.22E-06 to 5.37E-05)	0.14	4277	1.18E-05 (-2.55E-05 to 4.89E-05)	0.54	3449	3.51E-05 (-2.06E-05 to 9.08E-05)	0.22	1673	3.33E-05 (-3.27E-05 to 9.92E-05)	0.32	1116	5.56E-05 (-4.99E-05 to 1.16E-04)	0.30	425
MVPA	1.05E-05 (-5.07E-05 to 7.16E-05)	0.74	4277	-4.88E-06 (-8.12E-05 to 7.15E-05)	0.90	3449	-5.02E-05 (-1.88E-04 to 8.77E-05)	0.48	1673	-1.04E-04 (-2.57E-04 to 4.99E-05)	0.19	1116	-1.66E-04 (-4.02E-04 to 7.03E-05)	0.17	425
Boys															
ST	-7.03E-06 (-4.34E-05 to 2.93E-05)	0.71	2051	-1.27E-05 (-5.96E-05 to 3.42E-05)	0.60	1609	-2.41E-05 (-1.18E-04 to 6.93E-05)	0.61	676	-5.91E-06 (-1.17E-04 to 1.05E-04)	0.92	458	-1.23E-04 (-2.86E-04 to 3.92E-05)	0.14	169
LPA	2.46E-05 (-2.03E-05 to 6.95E-05)	0.28	2051	1.91E-05 (-3.66E-05 to 7.47E-05)	0.50	1609	2.41E-05 (-6.93E-05 to 1.17E-04)	0.61	676	5.92E-06 (-1.05E-04 to 1.17E-04)	0.92	458	1.23E-04 (-3.92E-05 to 2.86E-04)	0.14	169
MVPA	-1.85E-05 (-1.01E-04 to 6.43E-05)	0.66	2051	-3.81E-06 (-1.03E-04 to 9.59E-05)	0.94	1609	-7.74E-06 (-2.33E-04 to 2.18E-04)	0.95	676	-2.16E-05 (-2.74E-04 to 2.31E-04)	0.87	458	-3.49E-04 (-0.001 to 8.00E-05)	0.11	169
Girls															
ST	-2.98E-06 (-3.76E-05 to 3.16E-05)	0.87	2226	3.23E-06 (-4.70E-05 to 4.06E-05)	0.89	1840	-3.81E-05 (-1.07E-04 to 3.11E-05)	0.28	997	-5.04E-05 (-1.33E-04 to 3.17E-05)	0.23	658	-2.29E-05 (-1.67E-04 to 1.21E-04)	0.75	256
LPA	2.14E-05 (-2.00E-05 to 6.29E-05)	0.31	2226	4.95E-06 (-4.51E-05 to 5.50E-05)	0.85	1840	3.81E-05 (-3.11E-05 to 1.07E-04)	0.28	997	5.04E-05 (-3.17E-05 to 1.33E-04)	0.23	658	2.29E-05 (-1.21E-04 to 1.67E-04)	0.76	256
MVPA	2.60E-05 (-8.17E-05 to 1.34E-04)	0.64	2226	-4.30E-06 (-1.26E-04 to 1.17E-04)	0.95	1840	-7.70E-05 (-2.53E-04 to 9.92E-05)	0.39	997	-1.72E-04 (-3.67E-04 to 2.37E-05)	0.08	658	-7.65E-05 (-3.74E-04 to 2.21E-04)	0.61	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

Table A.9 – Associations between physical activity or sedentary time and arterial stiffness (observed data only) using compositional data analysis for accelerometer data

	Model 1 (unadjusted)			Model 2			Model 3			Model 4		
	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*	b $_{ilr1}$ (95% CI)	*P*-value	*n*
Group												
ST : LPA & MVPA	0.022 (-0.111 to 0.156)	0.75	4277	0.161 (-0.063 to 0.385)	0.16	1626	0.074 (-0.259 to 0.406)	0.66	779	0.073 (-0.363 to 0.509)	0.74	425
LPA : MVPA & ST	-0.131 (-0.302 to 0.040)	0.13	4277	-0.146 (-0.428 to 0.137)	0.31	1626	-0.149 (-0.551 to 0.254)	0.47	779	0.029 (-0.515 to 0.573)	0.92	425
MVPA : ST & LPA	**0.109 (0.017 to 0.201)**	**0.020**	4277	-0.016 (-0.173 to 0.142)	0.85	1626	0.075 (-0.159 to 0.309)	0.53	779	-0.102 (-0.411 to 0.206)	0.52	425
Boys												
ST : LPA & MVPA	0.079 (-0.114 to 0.272)	0.42	2051	0.151 (-0.169 to 0.471)	0.36	759	0.010 (-0.476 to 0.497)	0.97	318	0.139 (-0.531 to 0.809)	0.68	169
LPA : MVPA & ST	-0.214 (-0.466 to 0.039)	0.10	2051	-0.154 (-0.568 to 0.261)	0.47	759	-0.141 (-0.771 to 0.489)	0.66	318	0.115 (-0.799 to 1.018)	0.80	169
MVPA : ST & LPA	0.135 (-0.008 to 0.277)	0.06	2051	0.003 (-0.239 to 0.245)	0.98	759	0.131 (-0.272 to 0.533)	0.52	318	-0.253 (-0.832 to 0.325)	0.39	169
Girls												
ST : LPA & MVPA	-0.016 (-0.200 to 0.168)	0.86	2226	0.184 (-0.131 to 0.499)	0.25	867	0.138 (-0.326 to 0.601)	0.56	461	0.050 (-0.548 to 0.648)	0.87	256
LPA : MVPA & ST	0.062 (-0.176 to 0.299)	0.61	2226	-0.151 (-0.539 to 0.238)	0.45	867	-0.152 (-0.697 to 0.393)	0.58	461	0.009 (-0.714 to 0.733)	0.98	256
MVPA : ST & LPA	-0.046 (-0.181 to 0.090)	0.51	2226	-0.033 (-0.243 to 0.176)	0.75	867	0.014 (-0.283 to 0.312)	0.92	461	-0.059 (-0.440 to 0.321)	0.76	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class and the time between vascular and accelerometer measurement

Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index

Model 4 was adjusted for all in model 3, plus cardiometabolic risk score, and family history of hypertension, diabetes, high cholesterol and vascular disease

Table A.10 – Associations between physical activity or sedentary time and arterial stiffness (observed data) using a non-compositional multiple linear regression approach

	Model 1 (unadjusted)			Model 2			Model 3			Model 4			Model 5		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
Group															
ST	-1.68E-04 (-0.001 to 3.38E-04)	0.52	4277	3.46E-04 (-2.89E-04 to 0.001)	0.29	3449	-3.63E-05 (-0.001 to 0.001)	0.95	1673	-4.14E-04 (-0.002 to 0.001)	0.53	1116	3.16E-04 (-0.002 to 0.002)	0.77	425
LPA	-3.47E-04 (-0.001 to 2.70E-04)	0.27	4277	-4.11E-04 (-0.001 to 3.27E-04)	0.27	3449	3.71E-05 (-0.001 to 0.001)	0.95	1673	4.15E-04 (-0.001 to 0.002)	0.53	1116	-3.16E-04 (-0.002 to 0.002)	0.77	425
MVPA	**0.002 (3.22E-04 to 0.003)**	**0.014**	4277	-2.37E-04 (-0.002 to 0.001)	0.76	3449	0.001 (-0.001 to 0.004)	0.38	1673	3.62E-04 (-0.003 to 0.003)	0.82	1116	-0.001 (-0.006 to 0.004)	0.66	425
Boys															
ST	2.61E-05 (-0.001 to 0.001)	0.94	2051	0.001 (-3.72E-04 to 0.001)	0.24	1609	2.68E-04 (-0.001 to 0.002)	0.75	676	3.20E-04 (-0.002 to 0.002)	0.76	458	-1.12E-04 (-0.004 to 0.003)	0.95	169
LPA	-0.001 (-0.001 to 3.30E-04)	0.21	2051	-0.001 (-0.002 to 2.73E-04)	0.14	1609	-2.68E-04 (-0.002 to 0.001)	0.75	676	-3.20E-04 (-0.002 to 0.002)	0.76	458	1.11E-04 (-0.003 to 0.004)	0.95	169
MVPA	0.001 (-0.001 to 0.003)	0.18	2051	1.50E-04 (-0.002 to 0.002)	0.88	1609	0.002 (-0.002 to 0.006)	0.24	676	0.002 (-0.003 to 0.007)	0.45	458	-0.005 (-0.014 to 0.004)	0.29	169
Girls															
ST	1.59E-04 (-0.001 to 0.001)	0.66	2226	1.72E-04 (-0.001 to 0.001)	0.70	1840	-1.54E-04 (-0.002 to 0.001)	0.83	997	0.001 (-0.002 to 0.001)	0.39	658	3.77E-04 (-0.002 to 0.003)	0.80	256
LPA	-1.77E-04 (-0.001 to 0.001)	0.68	2226	-9.02E-05 (-0.001 to 0.001)	0.86	1840	1.55E-04 (-0.001 to 0.002)	0.83	997	0.001 (-0.001 to 0.002)	0.39	658	-3.76E-04 (-0.003 to 0.002)	0.80	256
MVPA	-1.31E-04 (-0.002 to 0.002)	0.91	2226	-0.001 (-0.003 to 0.002)	0.53	1840	0.001 (-0.003 to 0.004)	0.78	997	-2.46E-04 (-0.004 to 0.004)	0.91	658	-2.30E-04 (-0.006 to 0.006)	0.94	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

Table A.11 – Associations between physical activity or sedentary time and the clustered cardiometabolic risk score (observed data only) using compositional data analysis for accelerometer data

	Model 1 (unadjusted)			**Model 2**			**Model 3**			**Model 4**		
	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*	b *ilr1* (95% CI)	*P*-value	*n*
Group												
ST : LPA & MVPA	**0.169 (0.083 to 0.255)**	**<0.001**	2546	0.118 (-0.021 to 0.257)	0.10	1037	0.147 (-0.047 to 0.342)	0.14	521	0.158 (-0.069 to 0.384)	0.17	425
LPA : MVPA & ST	-0.044 (-0.154 to 0.067)	0.44	2546	-0.018 (-0.194 to 0.159)	0.84	1037	-0.004 (-0.245 to 0.236)	0.97	521	-0.032 (-0.315 to 0.251)	0.82	425
MVPA : ST & LPA	**-0.126 (-0.185 to -0.067)**	**<0.001**	2546	**-0.100 (-0.196 to -0.004)**	**0.041**	1037	**-0.143 (-0.281 to -0.006)**	**0.041**	521	-0.126 (-0.286 to 0.034)	0.12	425
Boys												
ST : LPA & MVPA	0.112 (-0.008 to 0.231)	0.07	1250	0.111 (-0.078 to 0.300)	0.25	501	0.216 (-0.077 to 0.509)	0.15	214	0.179 (-0.170 to 0.529)	0.31	169
LPA : MVPA & ST	0.070 (-0.088 to 0.228)	0.38	1250	-0.031 (-0.283 to 0.220)	0.81	501	-0.060 (-0.449 to 0.330)	0.76	214	-0.051 (-0.525 to 0.423)	0.83	169
MVPA : ST & LPA	**-0.182 (-0.271 to -0.092)**	**<0.001**	1250	-0.079 (-0.225 to 0.067)	0.29	501	-0.156 (-0.408 to 0.096)	0.22	214	-0.128 (-0.431 to 0.174)	0.40	169
Girls												
ST : LPA & MVPA	**0.236 (0.112 to 0.359)**	**<0.001**	1296	0.115 (-0.092 to 0.321)	0.28	536	0.071 (-0.196 to 0.337)	0.60	307	0.106 (-0.204 to 0.415)	0.50	256
LPA : MVPA & ST	-0.097 (-0.255 to 0.061)	0.23	1296	0.026 (-0.227 to 0.279)	0.84	536	0.080 (-0.240 to 0.399)	0.62	307	0.034 (-0.341 to 0.408)	0.86	256
MVPA : ST & LPA	**-0.139 (-0.166 to -0.025)**	**0.002**	1296	**-0.141 (-0.270 to -0.011)**	**0.034**	536	-0.150 (-0.320 to 0.019)	0.08	307	-0.139 (-0.334 to 0.056)	0.16	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity
Model 2 was adjusted for age at 9 year clinic, sex (in whole group models only), age in years from peak height velocity at 9 year clinic, mother's social class and the time between cardiometabolic risk assessment and accelerometer measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index
Model 4 was adjusted for all in model 3, and family history of hypertension, diabetes, high cholesterol and vascular disease

Table A.12 – Comparison of participant characteristics for the clustered cardiometabolic risk score and activity variables in model four using imputed data vs. listwise exclusion in compositional data analysis models

	Group		Boys		Girls	
	Imputed *n* = 4277	Listwise exclusion *n* = 425	Imputed *n* = 2051	Listwise exclusion *n* = 169	Imputed *n* = 2226	Listwise exclusion *n* = 256
Age at 9 year clinic	9.8 ± 0.3	9.8 ± 0.2	9.8 ± 0.3	9.8 ± 0.2	9.8 ± 0.3	9.8 ± 0.3
aPHV at 9 year clinic	-2.8 ±1.3	-2.7 ± 1.3	-3.7 ± 1.0	-3.7 ± 1.0	-2.0 ± 0.9	-2.0 ± 0.9
Mother's social class						
I (%)	5.7	7.1	6.6	11.2	4.8	4.3
II (%)	35.8	39.5	35.1	40.8	36.4	38.7
III (non-manual; %)	38.3	36.0	37.8	32.5	38.8	38.3
III (manual; %)	1.9	1.2	1.2	0.6	2.4	1.6
IV (%)	14.8	12.7	15.4	11.8	14.3	13.3
V (%)	3.6	3.5	3.8	3.0	3.3	3.9
Time between CMR score and accelerometer visits (y)	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.3	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.3
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$)	9.9 ± 1.4	9.9 ± 1.3	9.9 ± 1.4	10.3 ± 1.1	9.9 ± 1.4	9.6 ± 1.3
Lean mass index ($kg \cdot m^{-2}$)	12.54 ± 1.04	12.43 ± 0.85	12.96 ± 0.93	12.87 ± 0.73	12.16 ± 0.99	12.14 ± 0.81
Cardiometabolic risk score	0.06 ± 0.71	-0.01 ± 0.62	0.07 ± 0.69	0.05 ± 0.61	0.04 ± 0.71	-0.05 ± 0.62
Family history of CVD						
Yes (%)	30.3	29.2	29.7	27.8	30.8	30.1
No (%)	69.7	70.8	70.3	72.2	69.2	69.9
Accelerometer variables						
ST ($min \cdot day^{-1}$)	354.5 ± 72.6	360.4 ± 66.7	347.6 ± 73.6	353.9 ± 69.2	360.8 ± 71.2	364.6 ± 64.8
LPA ($min \cdot day^{-1}$)	366.5 ± 59.5	367.9 ± 59.0	367.4 ± 59.6	370.2 ± 62.8	365.6 ± 59.4	366.4 ± 56.4
MVPA ($min \cdot day^{-1}$)	57.6 ± 29.6	54.1 ± 29.0	68.2 ± 32.3	63.1 ± 6.2	47.7 ± 22.8	48.2 ± 29.2

Data presented as mean ± SD. aPHV = age in years from peak height velocity; PWC_{170} = peak work capacity at 170 beats per minute; ST = sedentary time; LPA = light physical activity; MVPA = moderate-vigorous physical activity.

Table A.13 – Associations between physical activity or sedentary time and the clustered cardiometabolic risk score (observed data) using a non-compositional multiple linear regression approach

	Model 1 (unadjusted)			**Model 2**			**Model 3**			**Model 4**			**Model 5**		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
Group															
ST	**0.001 (4.61E-04 to 0.001)**	**<0.001**	2546	**0.001 (3.39E-04 to 0.001)**	**<0.001**	2152	1.38E-04 (-4.82E-04 to 0.001)	0.66	1116	3.02E-04 (-3.00E-04 to 0.001)	0.33	1116	0.001 (-1.96E-04 to 0.002)	0.11	425
LPA	**-0.001 (-0.001 to -2.20E-04)**	**0.002**	2546	**-4.99E-04 (-0.001 to -4.17E-05)**	**0.033**	2152	-3.74E-04 (-0.001 to 8.52E-05)	0.11	1116	-3.01E-04 (-0.001 to 3.01E-04)	0.33	1116	-3.84E-04 (-0.001 to 0.001)	0.49	425
MVPA	**-0.002 (-0.003 to -0.001)**	**<0.001**	2546	**-0.002 (-0.003 to -0.001)**	**<0.001**	2152	**-0.002 (-0.003 to -2.61E-04)**	**0.021**	1116	**-0.002 (-0.003 to -2.58E-04)**	**0.020**	1116	-0.002 (-0.004 to 4.48E-04)	0.11	425
Boys															
ST	**0.001 (1.57E-04 to 0.001)**	**0.008**	1250	**0.001 (1.25E-04 to 0.001)**	**0.016**	1031	9.32E-05 (-0.001 to 0.001)	0.85	458	2.51E-04 (-0.001 to 0.001)	0.61	458	3.77E-04 (-0.001 to 0.002)	0.68	169
LPA	-4.10E-04 (-0.001 to 1.50E-04)	0.15	1250	-4.75E-04 (-0.001 to 1.85E-04)	0.16	1031	-9.29E-05 (-0.001 to 0.001)	0.86	458	-2.51E-04 (-0.001 to 0.001)	0.61	458	-3.75E-04 (-0.002 to 0.001)	0.68	169
MVPA	**-0.004 (-0.004 to -0.001)**	**<0.001**	1250	**-0.002 (-0.003 to -4.89E-04)**	**0.006**	1031	-0.001 (-0.004 to 0.001)	0.21	458	-0.001 (-0.004 to 0.001)	0.21	458	-0.003 (-0.007 to 0.002)	0.29	169
Girls															
ST	**0.001 (0.001 to 0.001)**	**<0.001**	1296	**0.001 (2.33E-04 to 0.001)**	**0.005**	1121	5.98E-05 (-0.001 to 0.001)	0.88	658	2.55E-04 (-0.001 to 0.001)	0.52	658	3.58E-05 (-0.001 to 0.002)	0.96	256
LPA	**-0.001 (-0.001 to -2.68E-04)**	**0.004**	1296	-4.94E-04 (-0.001 to 1.41E-04)	0.13	1121	-5.94E-05 (-0.001 to 0.001)	0.88	658	-2.55E-04 (-0.001 to 0.001)	0.52	658	-3.55E-05 (-0.002 to 0.001)	0.96	256
MVPA	**-0.003 (-0.004 to -0.001)**	**<0.001**	1296	**-0.003 (-0.004 to -0.001)**	**<0.001**	1121	**-0.002 (-0.004 to -3.36E-04)**	**0.021**	658	**-0.002 (-0.004 to -2.16E-04)**	**0.029**	658	-0.003 (-0.006 to 4.98E-04)	0.10	256

ST = sedentary time; LPA = light physical activity; MVPA = moderate to vigorous physical activity

Model 2 was adjusted for age at 9 year clinic, sex (in whole group models only), age in years from peak height velocity at 9 year clinic, the time between vascular and accelerometer measurement, and the accelerometer wear time

Model 3 was adjusted for all in model 2, plus an additional activity variable (MVPA when ST or LPA was the predictor variable; ST when MVPA was the predictor) and cardiorespiratory fitness scaled to lean body mass

Model 4 was adjusted for all in model 3, plus lean mass index and cardiometabolic risk score

Model 5 was adjusted for all in model 4, plus family history of hypertension, diabetes, high cholesterol and vascular disease and mother's social class

Table A.14 – Associations of vascular measures with the clustered cardiometabolic score (observed data only)

	Model 1 (unadjusted)			Model 2			Model 3		
	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*	b (95% CI)	*P*-value	*n*
FMD									
Group - CMR	0.116 (-0.102 to 0.333)	0.30	2546	**0.726 (0.391 to 1.062)**	**<0.001**	1037	**0.560 (0.056 to 1.065)**	**0.030**	521
Boys - CMR	0.019 (-0.273 to 0.311)	0.90	1250	**0.547 (0.066 to 1.026)**	**0.026**	501	0.409 (-0.357 to 1.174)	0.29	214
Girls - CMR	0.234 (-0.080 to 0.549)	0.14	1296	**0.902 (0.427 to 1.376)**	**<0.001**	536	**0.767 (0.086 to 1.449)**	**0.028**	307
DC									
Group - CMR	**-0.006 (-0.010 to -0.002)**	**0.002**	2546	**-0.010 (-0.016 to -0.004)**	**0.001**	1037	**-0.015 (-0.024 to -0.006)**	**0.001**	521
Boys - CMR	**-0.007 (-0.013 to -0.002)**	**0.011**	1250	**-0.013 (-0.021 to -0.004)**	**0.004**	501	**-0.020 (-0.034 to -0.006)**	**0.005**	214
Girls - CMR	-0.005 (-0.010 to 9.79E-05)	0.05	1296	**-0.008 (-0.017 to -1.74E-04)**	**0.045**	536	-0.010 (-0.022 to 0.001)	0.07	307
PWV									
Group - CMR	**-0.124 (-0.202 to -0.046)**	**0.002**	2546	-0.081 (-0.206 to 0.044)	0.20	1037	-0.027 (-0.199 to 0.145)	0.76	521
Boys - CMR	**-0.130 (-0.243 to -0.018)**	**0.023**	1250	-0.127 (-0.310 to 0.055)	0.17	501	-0.014 (-0.288 to 0.260)	0.92	214
Girls - CMR	**-0.123 (-0.231 to -0.014)**	**0.027**	1296	-0.025 (-0.199 to 0.149)	0.78	536	-0.042 (-0.268 to -0.183)	0.71	307

FMD = flow mediated dilation; PWV = pulse wave velocity; DC = distensibility coefficient; CMR = cardiometabolic risk score
Model 2 was adjusted for age at 10 year clinic, sex (in whole group models only), age in years from peak height velocity at 10 year clinic, mother's social class and the time between cardiometabolic risk score assessment and vascular outcome measurement
Model 3 was adjusted for all in model 2, plus cardiorespiratory fitness scaled to lean body mass and lean mass index

Table A.15 – Comparison of participant characteristics for vascular variables and clustered cardiometabolic risk score in model three using imputed data vs. listwise exclusion

	Group		Boys		Girls	
	Imputed *n* = 4277	Listwise exclusion *n* = 521	Imputed *n* = 2051	Listwise exclusion *n* = 214	Imputed *n* = 2226	Listwise exclusion *n* = 307
Age at 10 year clinic	10.6 ± 0.2	10.6 ± 0.2	10.6 ± 0.2	10.6 ± 0.18	10.6 ± 0.2	10.6 ± 0.18
aPHV at 10 year clinic	-2.0 ± 1.3	-2.0 ± 1.3	-2.9 ± 1.0	-3.0 ± 1.0	-1.2 ± 0.9	-1.2 ± 0.8
Mother's social class						
I (%)	5.7	6.0	6.6	8.9	4.8	3.9
II (%)	35.8	38.8	35.1	41.6	36.4	36.8
III (non-manual; %)	38.3	36.7	37.8	32.2	38.8	39.7
III (manual; %)	1.9	1.2	1.2	0.9	2.4	1.3
IV (%)	14.8	14.4	15.4	14.0	14.3	14.7
V (%)	3.6	3.1	3.8	2.3	3.3	3.6
Time between CMR score and vascular measures visits (y)	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.3
PWC_{170}·total body lean mass ($W \cdot kg^{0.585}$) at 9 year clinic	9.9 ± 1.4	9.9 ± 1.3	9.9 ± 1.4	10.3 ± 1.1	9.9 ± 1.4	9.7 ± 1.3
Lean mass index ($kg \cdot m^{-2}$) at 9 year clinic	12.54 ± 1.04	12.44 ± 0.86	12.96 ± 0.93	12.88 ± 0.77	12.16 ± 0.99	12.12 ± 0.78
Cardiometabolic risk score at 9 year clinic	0.06 ± 0.71	-0.00 ± 0.61	0.07 ± 0.69	0.05 ± 0.60	0.04 ± 0.71	-0.03 ± 0.61
Vascular variables						
Baseline vessel diameter (mm) age 10	2.66 ± 0.30	2.64 ± 0.29	2.75 ± 0.29	2.73 ± 0.29	2.58 ± 0.29	2.58 ± 0.27
FMD (%) age 10	8.13 ± 3.39	8.33 ± 3.53	7.75 ± 3.28	7.94 ± 3.33	8.48 ± 3.45	8.60 ± 3.66
DC (% per mmHg) age 10	0.12 ± 0.06	0.13 ± 0.06	0.13 ± 0.06	0.12 ± 0.06	0.12 ± 0.06	0.13 ± 0.06
PWV ($m \cdot s^{-1}$) age 10	7.56 ± 1.23	7.54 ± 1.17	7.64 ± 1.24	7.67 ± 1.17	7.48 ± 1.20	7.45 ± 1.17

Data presented as mean ± SD. aPHV = age in years from peak height velocity; PWC_{170} = peak work capacity at 170 beats per minute; CMR score = cardiometabolic risk score; FMD = flow mediated dilation; DC = distensibility coefficient and PWV = pulse wave velocity.