Title: Associations of sedentary behaviour patterns with cardiometabolic risk in children: the sit less for health study

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Associations of sedentary behaviour patterns with cardiometabolic risk in children: the sit less for health study

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Stephanie White 18th December 2017

Abstract

This study investigated the association between patterns of sedentary behaviour and cardiometabolic risk in children aged 11–12-years-old. Inclinometer and accelerometer determined sedentary behaviour patterns were measured in 118 (51 males) school children, in addition to cardiometabolic risk markers. Data were analysed using partial correlations and multiple linear regression. After adjustment for potential confounding variables, prolonged sedentary time was significantly negatively associated with weight (β=-.681), waist circumference (WC) (β=-.557), body mass index (BMI) (β=-.675) and body fat% (β=-.685) and significantly positively associated with total cholesterol (TC) (β=.410) and high-density lipoprotein cholesterol (HDL) (β=.432). The number of breaks in sedentary time was significantly negatively associated with weight (β=-.661), WC (β=-.597), BMI (β=-.601) and body fat% (β=-.546) and significantly positively associated with TC (β=.334) and HDL (β=.415). Total standing time was significantly negatively associated with weight (β=-.270), WC (β=-.272) and body fat% (β=-.286) and significantly positively associated with HDL (β=.312). This study provides evidence that the number of breaks in sedentary time and total standing time are beneficially associated with cardiometabolic risk in children aged 11–12-years-old. However, the associations of other sedentary behaviour variables cardiometabolic risk is mixed and thus requires further research.

Keywords: cardiometabolic risk; sedentary behaviour; standing; physical activity; children

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Publications to date

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Glossary

BMI – body mass index

CPM – counts per minute

CVD – cardiovascular disease

DEXA - Dual X-ray absorptiometry

DBP – diastolic blood pressure

HDL – high density lipoprotein cholesterol

LDL – low density lipoprotein cholesterol

MetS – metabolic syndrome

METs – metabolic equivalent tasks

MVPA – moderate to vigorous physical activity

Non-HDL – non-high-density lipoprotein

PE – physical education

SBP – systolic blood pressure

T2DM – type 2 diabetes mellitus

TC – total cholesterol

TC: HDL – total cholesterol to high density lipoprotein ratio

TRG: HDL – triglyceride to high density lipoprotein ratio

TV – television

US – United States

UK – United Kingdom

VLDL – very low-density lipoprotein

WC – waist circumference

1. Introduction

Type 2 Diabetes Mellitus (T2DM), traditionally associated with ageing, was first reported in children in the United Kingdom (UK) in the year 2000 (Ehtisham *et al.*, 2000) and its prevalence has continued to grow since (Reinehr, 2013). This can lead to complications with cardiovascular disease (CVD) in adulthood (Katzmarzyk *et al.*, 2001). CVD causes 26% of all deaths in the UK annually and there are approximately over 7 million people living with CVD in the UK (British Heart Foundation, 2016). Cardiometabolic risk is a condition where there is an increased risk of the development of T2DM and CVD (Isomaa *et al.*, 2001). Risk markers for cardiometabolic disease include obesity, hyperglycaemia, hypertension and dyslipidaemia, all of which can begin to manifest in childhood (Katzmarzyk *et al.*, 2001). This is problematic as cardiometabolic risk markers track from childhood to adulthood (Katzmarzyk *et al.*, 2001).

Sedentary behaviour is any waking behaviour characterised by an energy expenditure value of ≤ 1.5 Metabolic Equivalents (METs) whilst in a sitting or reclining posture (Sedentary Behaviour Research Network, 2012). Children in the UK spend between 7 – 8 hours a day in sedentary behaviour (Bailey *et al.*, 2012b). Previous studies have identified an association between sedentary time and cardiometabolic risk markers in children, however there are inconsistencies in their findings (Bailey *et al.*, 2016; Bailey *et al.*, 2014; Colley *et al.*, 2013; Martinez-Gomez *et al.*, 2010a; Saunders *et al.*, 2013a; Saunders *et al.*, 2013b; Tremblay *et al.*, 2011; Carson *et al.*, 2016a; Carson and Janssen, 2011; Altenburg *et al.*, 2015). Many of these studies objectively measured sedentary time using accelerometers that are unable to detect postural allocation. Therefore, standing could be misclassified as sedentary behaviour (Chastin and Granat, 2009).

This study aimed to use an activPAL inclinometer to accurately measure children's (aged 11 - 12 years old) sedentary behaviour patterns and investigate the association with cardiometabolic risk.

2. Literature review

2.1 Prevalence and economic burden of cardiometabolic disease

Cardiometabolic disease refers to the combination of CVD and diabetes (Fisher, 2006). The metabolic syndrome (MetS) refers to a clustering of risk markers for CVD and T2DM, including obesity, dyslipidaemia - high total cholesterol (TC), high triglycerides, high low-density lipoprotein cholesterol (LDL), low high-density lipoprotein cholesterol (HDL) - hypertension – high systolic blood pressure (SBP) and/or high diastolic blood pressure (DBP) - and hyperglycaemia – high fasting glucose concentrations (Alberti *et al.*, 2006; Zimmet *et al.*, 2007; Mahabaleshwarkar *et al.*, 2016). The greater number of risk markers an individual exhibits, the greater risk they have of developing T2DM and CVD, such as stroke, myocardial infarction, coronary heart disease, cerebrovascular disease, peripheral arterial disease (National Heart Lung and Blood Institute, 2016; World Health Organisation, 2016a).

CVD is the most common cause of death, representing 31% of deaths globally (World Health Organisation, 2016a). In 2012, an estimated 17.5 million people died from CVD, of which 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (World Health Organisation, 2016a). CVD causes over 158,000 deaths in the UK annually - 26% of all deaths - and there are an estimated 7 million or more people living with CVD in the UK, which costs an estimated £15 billion per year (British Heart Foundation, 2016).

It is predicted that ischemic heart disease will be the leading cause of death globally in 2030, followed by cerebrovascular disease and diabetes (Mathers and Loncar, 2006). Most CVDs can be prevented through healthy behaviours, such as a healthy diet, not smoking, maintaining a healthy weight, avoiding harmful alcohol use, and being physically active (World Health Organisation, 2016a). CVD is

rarely found in children and adolescence; however, children can still present with CVD risk markers and over time this may lead to the development of CVD in adulthood.

In the UK it is estimated that 4 million people are currently living with diagnosed diabetes, of which 90% are T2DM, with a further 519,000 undiagnosed cases (Diabetes U.K., 2015). The global prevalence of diabetes in 2014 was 8.5%, 422 million people (World Health Organisation, 2016b). An estimated 1.5 million deaths in 2012 were directly caused by diabetes and a further 2.2 million were attributable to hyperglycaemia, globally (World Health Organisation, 2016b). It is estimated that 10% of the NHS budget is spent on diabetes, which is approximately £9.8 billion per year (Hex *et al.*, 2012).

2.2 Cardiometabolic disease in children

Cardiometabolic disease is an uncommon occurrence or cause of death in children; however, risk markers can begin to develop in childhood, increasing the likelihood of developing cardiometabolic disease in adulthood (Kavey *et al.*, 2003; Morrison *et al.*, 2007). Risk markers for cardiometabolic disease can begin to manifest in childhood (Katzmarzyk *et al.*, 2001). A clustering of these risk markers in childhood and adulthood, also known as the metabolic syndrome (MetS), has been shown to increase risk of developing T2DM and/or CVDs (Isomaa *et al.*, 2001). Morrison *et al.* (2007) concluded that children with MetS were at significantly greater risk of developing adult metabolic syndrome and T2DM than children without MetS.

The odds of a child becoming an overweight or obese adult increased by 8.1% each year between 1994 – 2003 in the UK (van Jaarsveld and Gulliford, 2015). Between 2003 – 2013 childhood obesity rates stabilised, with the odds of a child becoming an overweight or obese adult only increasing by 0.4% per year (van Jaarsveld and Gulliford, 2015). However, it is likely that this 'stabilisation' is rather a 'ceiling'

effect as prevalence is so high (Olds *et al.*, 2011) or that prevalence of obesity is not increasing but the degree of obesity in affected children may be (May *et al.*, 2012).

2.2.1 Type 2 diabetes mellitus in children

T2DM can be characterised by impaired fasting glucose and can be defined as a fasting glucose concentration ≥ 5.6 mmol · L^{-1} in children (Zimmet *et al.*, 2007). T2DM was traditionally associated with ageing until the first cases of T2DM in children were reported in the UK in 2000 (Ehtisham *et al.*, 2000). Between 2002 – 2003 and 2011 – 2012 in the US, cases of T2DM in children aged 10 – 19 years old increased from 9.0 cases per 100,000 youths per year to 12.5, which is an increase of 7.1% per year (Mayer-Davis *et al.*, 2017). Approximately 2.2% of all diabetes cases in children and young people (< 24 years old and under the care of a consultant paediatrician) in the UK are now T2DM (Royal College of Paediatrics and Child Health, 2017). According to the Royal College of Paediatrics and Child Health (2017), of the children and young people with T2DM in England and Wales, 30.6% live in the London and South East area, highlighting that there are regional differences in the prevalence of T2DM in children.

The increase in T2DM in children and youth is still on the rise, possibly due to increasing obesity prevalence (Reinehr, 2013), which is alarming as this increases risk and accelerates the development of cardiovascular complications in adulthood (Duncan, 2006). Adolescents aged 10 – 17 years old (n = 704, 65% female) in the TODAY study suffered with T2DM; 14% also suffered hypertension defined as ≥ 95^{th} percentile, 80% demonstrated low HDL (females < 50 mg/dl, males < 40 mg/dl) and 10% had hypertriglyceridemia (≥ 200 mg/dl) (Copeland *et al.*, 2011). This suggests a connection between T2DM and CVD risk markers in children and adolescence.

2.2.2 Atherosclerosis in children

Atherosclerosis was first discovered in children from casualties of the Korean and Vietnam Wars (National Heart Lung and Blood Institute, 2012). Body mass index (BMI), SBP and DBP, TC, triglycerides, LDL and HDL, as a group have been strongly associated with the extent of the lesions in the aorta and coronary arteries in persons aged 2 – 39 years old (Berenson *et al.*, 1998). Pathological studies have shown atherosclerotic lesions at autopsy after unexpected death of children correlate positively and significantly with risk markers such as LDL, triglycerides, SBP, DBP and BMI (Kavey *et al.*, 2003). The presence of fatty streaks and fibrous plaques in the aorta and coronary arteries increases with age; fatty streaks in the coronary arteries increased from ~50% at age 2 – 15 years old to ~85% age 21 – 39 years old (Berenson *et al.*, 1998). The extent of the fatty streak lesions in the coronary arteries was 8.5 times as great in persons with 3 or 4 risk markers as those with none and the extent of fibrous plaque lesions in the coronary arteries was 12 times as great (Berenson *et al.*, 1998). Participants with increased number of risk markers, such as obesity, adverse lipid profile or hypertension, had an increase in surface area of the fatty streaks in the aorta: 1 risk marker = 19.1%, 2 risk markers = 30.3%, 3 risk markers = 37.9% and 4 risk markers = 35% (Berenson *et al.*, 1998). This is a problem because the presence of atherosclerosis in childhood significantly increases the risk of CVD and interventions to attenuate these risk markers are therefore important.

2.3 Risk markers for cardiometabolic disease in children

2.3.1 Overweight and obesity in children

Adiposity can be defined using BMI, body fat% and waist circumference (WC). Measuring BMI to assess adiposity, overweight is defined as a BMI ≥ 85th percentile and < 95th percentile and obesity is defined as a BMI ≥ 95th percentile for children (Centres for Disease Control and Prevention, 2016). Using body

fat% to assess adiposity, after adjustments for age and sex, overweight ≥ 85th and obese ≥ 95th obese percentiles (McCarthy *et al.*, 2006). Using WC to assess adiposity, obese ≥ 90th percentile for age and sex (McCarthy *et al.*, 2001). Obesity is a marker for cardiometabolic disease risk and is often linked to other cardiometabolic risk markers (Katzmarzyk *et al.*, 2001) and plays a key role in the development of the metabolic syndrome (Molnar, 2004). Cardiometabolic risk factors are more prevalent in obese children than non-obese children and risk markers tend to cluster in obese children (Reilly *et al.*, 2003).

The global age-standardised prevalence of obesity has risen in girls from 0.7% in 1975 to 5.6% in 2016 and in boys from 0.9% in 1975 to 7.8% in 2016 (Abarca-Gómez *et al.*, 2017). The National Child Measurement Programme for England 2015/16 found that 34.2% of children aged 10 – 11 years old and 22.1% of children aged 4 – 5 years old were overweight or obese (National Statistics, 2016b). There are currently more children aged 10 – 11 years that are obese than overweight; in girls 17.9% are obese and 14.3% overweight and in boys 21.7% are obese and 14.3% overweight (National Statistics, 2016b). In 11 – 12 year olds, 20% of boys and 14% of girls were found to be obese (National Statistics, 2016a). This is concerning and in August 2016 the Government published the 'Childhood Obesity: A plan for action' strategy detailing how the Government aims to significantly reduce the rate of childhood obesity in England over the next decade (H M Government, 2016).

In a systematic review Friedemann *et al.* (2012) found that children, aged 5 – 15 years old, with a BMI outside of the normal range had significantly increased cardiometabolic risk than children within normal BMI range. They found SBP was greater by 4.54 mmHg in overweight and 7.49 mmHg in obese children compared with normal weight children. In addition, blood lipids were adversely associated in obese compared to normal weight children; TC was 0.15 mmol · L^{-1} higher and triglycerides 0.26 mmol · L^{-1} higher in obese children, fasting insulin and insulin resistance was only significantly higher in obese

children, not overweight children (Friedemann *et al.*, 2012). Similarly, overweight children (≥ 85th percentile) aged 5 – 10 years old have a significant increased odds ratio (OR) compared with children < 85th percentile for raised DBP (OR 2.4), raised SBP (OR 4.5), raised LDL (OR 3.0), low HDL (OR 3.4), raised triglycerides (OR 7.1) and high fasting insulin concentration (OR 12.1); 58% of these overweight children had one of these cardiometabolic risk markers and 25% had two or more (Freedman *et al.*, 1999). Wiklund *et al.* (2017), using DEXA, found that children with normal body weight and BMI but with a high body fat% had significantly higher cardiometabolic risk compared with children with normal weight and low percent body fat. The authors also found that participants who were overweight or obese in adulthood had a higher BMI by age 4, suggesting that body fat% could indicate a risk factor for cardiometabolic disease regardless of body weight. This highlights the importance of using body composition, not just weight, when assessing cardiometabolic disease risk in children.

2.3.2 Lipids in children

High TC, high LDL and low HDL are correlated with the extent of early atherosclerotic lesions in adolescents (National Cholesterol Education Program Expert Panel on Blood Cholesterol Levels in Children and Adolescents, 1992). TC and LDL were significantly correlated with fatty streaks in the aorta and coronary arteries and LDL with fibrous plaques in the coronary arteries from people who died between the ages of 2 and 39 years old (Berenson *et al.*, 1998). Newman *et al.* (1986) found associations between aortic fatty streaks and LDL ($r = 0.67$, $p < 0.0001$) independent of race, sex and age, and were inversely correlated with the ratio of HDL: LDL ($r = -0.35$, $p = 0.06$). Increasing levels of LDL during adolescence increases the risk of T2DM in women but may reduce the risk in men (Pollock *et al.*, 2017), indicating that there are differences between the sexes.

Non-HDL levels (TC minus HDL) ≥ 7.2 mmol · L^{-1} have been associated with increased odds of insulin resistance and MetS however triglyceride to HDL ratio (TRG: HDL) levels ≥ 2.2 have been associated with increased odds for insulin resistance, high blood pressure, MetS and preclinical signs of organ damage, as compared with children with non-HDL ≥ 7.2 mmol · L^{-1} (Di Bonito *et al.*, 2015). TRG: HDL may also be a useful indicator of impaired glucose tolerance in overweight and obese children aged 5 – 10 years old (Manco *et al.*, 2016) and 10 – 14 years old (Bailey *et al.*, 2014). Thus, it may be important to consider TRG: HDL to identify children who are at risk of developing cardiometabolic disease. This suggests that analysing the blood lipid profile could provide an insight to a child's risk of developing cardiometabolic disease and potential response to risk reducing interventions.

2.3.3 Blood pressure in children

Hypertension in children and adolescence is defined as SBP and/or DBP ≥ 95th percentile for the child's age and sex; pre-hypertension is defined as SBP and/or DBP between 90th – 95th percentile for the child's age and sex (National Heart Lung and Blood Institute, 2004). Hypertension and pre-hypertension are often undiagnosed in paediatric populations, potentially due to a lack of knowledge of normal blood pressure ranges in children as it is a function of age, sex and height percentile or due to a lack of awareness of previous readings (Hansen *et al.*, 2007). Andrade *et al.* (2010) suggests a prevalence of hypertension in children and adolescence of 2 – 5%. Hansen *et al.* (2007) determined the prevalence of hypertension in 3 – 18 year olds (n = 14187) to be 3.6% and 3.5% for pre-hypertension. Rosner *et al.* (2013) found the prevalence of pre-hypertension and hypertension in children aged 12 – 19 years old (n = 11636, males = 5,750) from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999 – 2008, to be 19.2% for boys and 12.6% for girls. In 10 – 14 year old children in the UK (n = 111), prevalence of elevated SBP has been shown to be as high as 18.9% and elevated DBP 18% (Bailey *et al.*, 2016).

Rates of hypertension in children and adolescence (aged 8 – 17 years old) in the United States (US) increased between the years of 1988 – 2000, SBP by 1.4 mm Hg and DBP by 3.3mm Hg, and has been partially attributed to an increase in the prevalence in overweight and obesity (Muntner *et al.*, 2004). McNiece *et al.* (2007) found the prevalence of hypertension in adolescence (n = 6790) to be 3.2% and the prevalence of pre-hypertension to be 15.7%, although over 30% of boys and 23 – 30% of girls in this sample were obese. This indicates a strong relationship between body weight, body composition and blood pressure, which is also supported in other studies (Goldfield *et al.*, 2011; Karatzi *et al.*, 2017).

Increased blood pressure values in childhood has been associated with atherosclerotic development (Berenson *et al.*, 1998; Lurbe, 2003). Berenson *et al.* (1998) conducted autopsies on young persons aged 2 – 39 years old and found strong positive correlations between systolic blood pressure and the extent of fatty streaks in the aorta and coronary arteries. Strong positive correlations were found between fibrous plaques in the coronary arteries but not in the aorta; and strong positive correlations were also found between diastolic blood pressure and the extent of fibrous plaques in the coronary arteries (Berenson *et al.*, 1998). This suggests that elevated blood pressure in childhood is associated with atherosclerotic development. Karatzi *et al.* (2017) found a prevalence of pre-hypertension in Greek children aged 9 – 13 years old (n = 2571) to be 14.2% and the prevalence of hypertension was 23%; a greater prevalence of hypertension was demonstrated in girls (25.3%) compared to boys (20.8%). Karatzi *et al.* (2017) suggest the hypertensive phenotype mostly represented in childhood is the isolated systolic hypertension, with a prevalence of 6.9% in 9-year-old children and 14.1% in 13-year-old children. Research has shown that children aged 5 – 14 years old (n = 1505, 56% female) that have a blood pressure reading in the top quintile continue to have high blood pressure as adults >15 years later, even after controlling for BMI; of the adults that developed hypertension, 48% suffered

elevated childhood SBP and 41% elevated childhood DBP (Bao *et al.*, 1995). Falkner *et al.* (2008) found that among adolescents with pre-hypertension (n=1470), 14% had developed hypertension two years later. The authors also found that children with high risk BP values in the top quintile, 63% of boys and 43% of girls had developed hypertension or pre-hypertension two years later. Pollock *et al.* (2017) found that boys with a greater SBP are at greater risk of T2DM as men; however, SBP had no association with the development of T2DM in women. Chen and Wang (2008) conducted a systematic review of 50 cohort studies and suggested that blood pressure tracking from childhood to adulthood is strong, thus early interventions for lowering blood pressure and maintaining a healthy blood pressure are important.

2.3.4 Metabolic syndrome in children

MetS comprises a cluster of cardiometabolic disease risk factors, including hypertension, abnormal glucose metabolism, dyslipidaemia, and abdominal obesity (Weiss *et al.*, 2013). Children considered to have cardiometabolic disease risk as characterised by the MetS have more than three of the following cardiometabolic risk factors: high WC, elevated triglycerides, elevated systolic or diastolic BP, impaired fasting glucose, and low HDL concentrations (Zimmet *et al.*, 2007). Weiss *et al.* (2004) found that the MetS was becoming more common among children and adolescents and that its prevalence directly increases with the degree of obesity. The NHANES study (n = 2456) reported a MetS prevalence of 8.6% in children and adolescents aged 12 – 19 years old in the US (10.8% males, 6.1% females) (Johnson *et al.*, 2009). In a systematic review, the prevalence of MetS in children and adolescence ranged from 1.2% to 22.6%, with samples using overweight and obese participants reporting rates up to 60% (Tailor *et al.*, 2010). Magge *et al.* (2017) highlights that researchers using the same database report varying prevalence's of MetS in American adolescence, from 4.2% (Cook *et al.*, 2003) to 9.2% (de Ferranti *et al.*, 2004). The variance in prevalence of MetS in children suggests a

universal definition of MetS is required to be used and future research should report prevalence of MetS to add to the current literature.

Huang *et al.* (2015) suggest that the assessment of cardiometabolic risk in childhood and adolescence is important as it may help define early causal factors and characterise preventative measures. Cardiometabolic risk markers track from childhood to adulthood (Katzmarzyk *et al.*, 2001), therefore lifestyle behaviours that are associated with these risk markers need to be identified in order to inform the development of public health interventions.

2.4 Sedentary behaviour patterns in children

Sedentary behaviour is any waking behaviour characterised by an energy expenditure value of ≤ 1.5 Metabolic Equivalents (METs) whilst in a sitting or reclining posture (Sedentary Behaviour Research Network, 2012; Tremblay *et al.*, 2017). Throughout the general population sedentary behaviour is increasing as advances in technology improve workplace productivity, transportation, communication and domestic entertainment (Bailey, 2015).

Children in Europe aged 10 – 14 years old demonstrate approximately 7 – 8 hours of objectively measured sedentary time each day (Bailey *et al.*, 2012a; Verloigne *et al.*, 2013) and children in the UK spend up to 80% of their waking day being sedentary (Basterfield *et al.*, 2011). Bailey *et al.* (2016) used a triaxial accelerometer to measure the sedentary time of children aged 10 – 14 years old over seven days during waking hours and found that participants engaged in an average of seven prolonged sedentary bouts (≥ 20 min) per day lasting an average of 37 minutes each. Other studies have shown on average that 8 – 11 year olds engage in five sedentary bouts per day of 15 – 29 minutes (Saunders *et al.*, 2013b) and that children aged 10 – 13 years olds engage in only two sedentary bouts ≥ 20

minutes per day (Altenburg *et al.*, 2015). Studies vary as to the definition of sedentary behaviour bouts with some defining a prolonged sedentary bout as 30 minutes or more of sedentary time but allowing six individual interruptions lasting approximately one minute each (Carson and Janssen, 2011; Colley *et al.*, 2013). In addition, the definition of 'breaks in sedentary time' varies between periods of more than 5 s (Carson *et al.*, 2014) up to 60 s (Saunders *et al.*, 2014). Altenburg *et al.* (2015) reported total time in sedentary bouts ≥ 20 minutes was 146 min/day using a 60s tolerance of non-sedentary behaviours in sedentary bouts, 105 min/day using a 30s tolerance and 56 min/day using a zero tolerance. The authors also report time spent in prolonged sedentary bouts ≥ 30 min using a 60s tolerance was 80min/day, 30s tolerance was 57 min/day and zero tolerance was 32 min/day. This suggests that even the same data set demonstrating patterns of sedentary behaviour can be interpreted using different definitions and this may cause problems when comparing studies and when associating sedentary patterns with cardiometabolic risk markers. Altenburg *et al.* (2015) suggest more significant associations between cardiometabolic risk factors and sedentary time is found when applying the strictest definition of uninterrupted sedentary time, which is zero tolerance of a break in sedentary time. It is therefore important to define what constitutes a 'sedentary bout' and what constitutes a 'break in sedentary time' for each study as this may affect associations found with cardiometabolic risk markers (Tremblay *et al.*, 2017).

Sedentary time differs between age groups and sexes. A systematic review of studies that objectively measured sedentary time in 27, 637 children from 10 different countries observed that as age increased, volume of sedentary time increased at an almost equivalent 'displacement' of light-intensity physical activity (Cooper *et al.*, 2015). Between Year 1 (age 5 – 6) and Year 4 (age 8 – 9) boys time spent sedentary increased by 20% from 354 to 428 minutes per day, and for girls by 23% from 365 to 448 minutes per day (Jago *et al.*, 2017). A greater increase in sedentary time has been shown

in children aged between 9 – 12 years old, compared to 7 – 9 year olds and 12 – 15 year olds, indicating that this population are susceptible to an increase in prolonged sedentary bouts (Mann *et al.*, 2017). A similar increase during adolescence was found by Janssen *et al.* (2016), from 51% of waking hours spent sedentary aged 7 years old to 74% at age 15 years old, with the steepest increase occurring between ages 9 – 12 years; the authors also found that the number of breaks per hour of sedentary time decreased from 8.6 to 4.1 breaks per hour between the age groups 7 – 9 years old and 12 – 15 years old. Bailey *et al.* (2016) found children aged 10 – 14 years old engaged in an average of 63 breaks in sedentary time per day, however Colley *et al.* (2013) found the number of breaks in 6 – 19 year old children was 81 in boys and 85 in girls. These studies indicate that the age group between 9 – 12 years old are highly susceptible to increases in total sedentary time and a reduction in breaks in sedentary time, suggesting an increase in time spent in prolonged sedentary bouts.

2.5 Associations of sedentary behaviour patterns with cardiometabolic risk in children

Research in sedentary behaviour and cardiometabolic risk in children is still in its infancy and the limited numbers of studies that objectively measure sedentary behaviour patterns in children available provide contradictory results (Colley *et al.*, 2013; Altenburg *et al.*, 2015; Carson and Janssen, 2011; Saunders *et al.*, 2013a; Bailey *et al.*, 2016). Carson and Janssen (2011) found that despite children and adolescents spending 51% of their waking hours in sedentary time, the volume of sedentary time was not an independent predictor of cardiometabolic risk, suggesting confounding variables, such as the pattern or type of sedentary time, may influence risk. No significant associations between accelerometer-measured sedentary time and cardiometabolic health risk was found in children aged

6 – 19 years old (Colley *et al.*, 2013). No association was found between total sedentary time and adverse cardiometabolic risk markers, such as body composition, blood pressure, triglycerides, HDL, LDL, TC or glucose, in children aged 11 – 12 years old (Stamatakis *et al.*, 2015). Altenburg *et al.* (2015) found no evidence for an association between the bouts of prolonged sedentary time and cardiometabolic risk markers in 10 – 13-year-old children. The duration of a prolonged sedentary bout were not significantly associated with cardiometabolic risk in children aged 10 – 14 years old (Bailey *et al.*, 2016). The number of breaks in sedentary time in 8 – 11 year old children was negatively associated with a clustered cardiometabolic risk score and BMI Z-scores in both sexes (all $p < 0.05$)(Saunders *et al.*, 2013b). The number of breaks in sedentary time were not significantly associated with cardiometabolic risk in children aged 10 – 14 years old (Bailey *et al.*, 2016).

The studies mentioned above all used accelerometers that cannot distinguish differences in posture and thus may be incorrectly classifying standing as sedentary time, which must be in a sitting or reclining posture (Tremblay *et al.*, 2017). Many of the above studies use an accelerometer cut off point of < 100 cmp to define sedentary time, however Stamatakis et al. (2015) used a higher value of < 200 cmp. The null-associations found between sedentary time and cardiometabolic risk markers by Stamatakis *et al.* (2015) compared to the other studies may be due to the higher cut off point as it may also include some light physical activity behaviours. In addition, blood samples were taken 3 – 5 years post accelerometer data collection by Stamatakis *et al.* (2015), meaning that the participants may have changed their sedentary behaviour patterns during this time which may have influenced their cardiometabolic risk marker levels. Studies objectively measuring sedentary behaviour patterns in children should therefore use inclinometers to account for postural differences (Atkin *et al.*, 2012).

2.5.1 Obesity

Sedentary behaviour has been associated with adiposity in adolescents, irrespective of dietary intake (Fletcher *et al.*, 2015). Total sedentary time has been significantly negatively correlated with abdominal adiposity in children aged 10 – 14 years old (Bailey *et al.*, 2016). Breaks in sedentary time and the number of 1 – 4 min sedentary bouts have been negatively associated with BMI z-score in children aged 8 – 11 years old and the quantity of sedentary bouts lasting 5 – 9 minutes has been negatively associated with WC in girls aged 8 – 11 years but not in boys of the same age (Saunders *et al.*, 2013b). The authors also reported a significant positive association between sedentary bouts lasting 10 – 14 minutes with BMI-Score in boys. Colley *et al.* (2013) found that prolonged bouts of sedentary time after 3pm lasting at least 40 minutes were positively associated with waist circumference and bouts of at least 80 minutes were positively associated with BMI and waist circumference in boys aged 11 – 14 years, but not in girls of the same age. Each additional 60 minutes of sedentary time accumulated during the after school period was associated with a 1.4 $kg \cdot m^{-2}$ higher BMI and a 3.4 cm waist circumference in 11 – 14 year old boys, but not in girls (Colley *et al.*, 2013). This could suggest that sedentary patterns after 3pm could be important for cardiometabolic risk in children. Increased sedentary time between the ages of 7 – 15 years old has been associated with increased adiposity; specifically, BMI and fat mass (Mann *et al.*, 2017). Despite BMI and WC being positively associated with prolonged bouts of sedentary time in boys aged 11 – 14 years, there was no associated risk between accelerometer measured sedentary time and cardiometabolic risk markers in boys and girls aged 6 – 19 years old, specifically blood pressure and non-HDL (Colley *et al.*, 2013). Sedentary behaviour patterns appear to be associated with adiposity in previous studies, thus it is important to consider different measures of adiposity when investigating associations between cardiometabolic risk markers and sedentary behaviour patterns in children.

2.5.2 Glucose

Sedentary bouts lasting 10 – 14 minutes have been positively associated with fasting glucose in girls aged 8 – 11 but not in boys of the same age, indicating differences between the sexes (Saunders *et al.*, 2013b). Children aged 13 – 17 years, split into tertiles of low, medium and high sedentary time, demonstrated a significant difference in fasting glucose concentrations between the low sedentary (91 mg/dL) and high sedentary (94.8 mg/dL) groups (Martinez-Gomez *et al.*, 2010b). In a systematic review of studies, limited evidence was found to support an association between objectively measured sedentary behaviour patterns and glucose (Fröberg and Raustorp, 2014). Other studies also report no significant associations between sedentary behaviours and fasting glucose (Chaput *et al.*, 2013; Chinapaw *et al.*, 2012; Hsu *et al.*, 2011). The inconsistency found in the associations between glucose and sedentary behaviour indicates more research is required.

2.5.3 Lipids

Boys have been shown to have higher concentrations of HDL and triglycerides than girls despite only demonstrating a significant difference in the number of 1 – 4 minute bouts of sedentary time (boys = 67, girls = 70) and no significant difference between sedentary bouts of 5 – 9 minutes, 10 – 14 minutes, 15 – 29 minutes or 30 + minutes (Saunders *et al.*, 2013b). This suggests that reducing time spent in sedentary bouts may be beneficial to cardiometabolic risk markers and that boys tend to spend greater time in short sedentary bouts than girls. Cliff *et al.* (2014) found that overall sedentary time was inversely associated with HDL in overweight/obese children aged 5 – 10 years old, independent of MVPA or WC. Similarly, Hjorth *et al.* (2014) found a higher duration of sedentary time was significantly associated with lower HDL in Danish children aged 8 – 11 years old. No association was found between total sedentary time, breaks in sedentary time or prolonged sedentary bouts of 20, 40, 60, 80, 100 and 120 minutes and non-HDL cholesterol in boys or girls aged 11 – 14 years old (Colley *et al.*, 2013). The

mean duration of daily breaks in sedentary time was significantly negatively correlated with TC (Bailey *et al.*, 2016) indicating that breaking up sedentary time is beneficial for TC levels. Resting, assessed by questionnaire but validated by a monitor combining HR and accelerometery, has also been significantly associated with VLDL and LDL cholesterol, triglycerides and cardiometabolic risk score in 6-8-year-old children in Finland when adjusted for age, sex and total PA (Vaisto *et al.*, 2014). The blood lipid profile appears to be affected by the duration of sedentary bouts, total sedentary time and breaks in sedentary time, thus should be considered when investigating associations of sedentary behaviour patterns and cardiometabolic risk in children.

Children aged 10 – 14 years old, who engaged in more prolonged sedentary bouts, had a higher odds of hypertriglyceridemia and increased clustered cardiometabolic risk, independent of abdominal obesity (Bailey *et al.*, 2016), suggesting prolonged sedentary behaviour detrimentally affects cardiometabolic risk markers in children. However, weak but significant negative associations have been found between bouts of sedentary behaviour lasting longer than 30 minutes and triglycerides in children aged 8 – 11 years old (Altenburg *et al.*, 2015) and the number of sedentary bouts lasting 15 – 29 minutes was negatively associated with fasting triglycerides in boys only (Saunders *et al.*, 2013b). Total sedentary time has been significantly positively associated with triglyceride levels in children aged 6 – 8 years old in Finland, however, this was no longer significant after adjustment for total physical activity, the number of meals consumed per day or body fat percentage (Vaisto *et al.*, 2014); Martinez-Gomez *et al.* (2010b) found a significant difference in triglycerides in children aged 13 – 17 years old who engaged in low amounts of sedentary behaviour (59.9 mg/dL) and high amounts of sedentary behaviour (68 mg/dL). These studies suggest there is inconclusive evidence for the associations between patterns of sedentary behaviour and lipid profile in children, and there may be sex and age factors that affect this, therefore requires further investigation.

2.5.4 Blood pressure

Sedentary time has been positively associated with diastolic BP in children 8 – 10 years old with at least one obese parent, however, the association was no longer statistically significant after adjusting for MVPA. After adjusting for sex, ethnicity, total sedentary time, MVPA and accelerometer wear time, Bailey *et al.* (2016) found that the duration of prolonged sedentary bouts was significantly positively correlated with SBP. Significant differences were found in SBP between children who engaged in low levels of sedentary time (123.1 mmHg) and those who engaged in high levels of sedentary time (129.3 mmHg) (Martinez-Gomez *et al.*, 2010b). A positive association was also found between sedentary behaviour and SBP and DBP in children aged 9 years old and 15 years old (Ekelund *et al.*, 2007). This suggests that spending time in prolonged sedentary bouts adversely affects blood pressure in children. However, many studies have found no significant associations between SBP and DBP with sedentary behaviour (Chaput *et al.*, 2013; Colley *et al.*, 2013; Hsu *et al.*, 2011; Carson and Janssen, 2011). This indicates that the relationships between blood pressure and sedentary behaviour patterns requires further research.

2.6 Limitations of accelerometer—determined sedentary behaviour patterns in previous studies

To date, many accelerometer studies conducted with children classify sedentary behaviour without being able to detect postural differences, therefore standing could be misclassified as sedentary time (Altenburg *et al.*, 2015; Carson and Janssen, 2011; Carson *et al.*, 2014; Saunders *et al.*, 2013a; Cooper *et al.*, 2015). This is problematic and can lead to overestimations of sedentary time which may affect the observed associations with health outcomes (Janssen *et al.*, 2016). Studies using accelerometers

with a 1-min epoch, such as Bailey *et al.* (2016), also present the possibility of the misclassification of sedentary time due to children's sporadic and intermittent behaviour (Carson *et al.*, 2014), therefore using an inclinometer may be more appropriate. Aguilar-Farias *et al.* (2015) found that pre-school children aged 4 – 5 years old accumulated 50% of their total daily steps in less than 100 steps per minute bouts and approximately 60% of these were accumulated in intervals of less than 10 seconds, which suggests that accelerometers that define sedentary behaviour as <100 cpm may be misclassifying active behaviours with little movement as sedentary. Therefore studies investigating sedentary behaviour patterns in children should use inclinometers to detect postural changes and more accurately measure sedentary behaviour (Atkin *et al.*, 2012).

2.6.1 Investigating sedentary behaviour patterns in children using inclinometers

The activPAL device is a small and lightweight inclinometer, worn on the mid-thigh on the right leg, that uses accelerometer-derived information about thigh position to estimate time spent in different postures in 15 second epochs; a horizontal position suggests lying or sitting and a vertical position suggests standing (Kozey-Keadle *et al.*, 2011). The activPAL device can accurately distinguish between sitting/lying, standing, stepping and the transition between sitting to standing in children and, unlike pedometers, does not misclassify fidgeting as steps taken (Aminian and Hinckson, 2012). This is due to the software algorithm that only counts events longer than a specified duration, which is 10 seconds at the manufacturer default, thus a minimum period of 10 seconds is required to define a new posture (Alghaeed *et al.*, 2013). There are currently no studies that have objectively measured sedentary behaviour patterns and associations with cardiometabolic risk in children using inclinometers, thus this needs to be explored.

2.7 Aim and Hypothesis

The aim of this study was to use an activPAL inclinometer to accurately measure children's (aged 11 – 12 years old) sedentary behaviour patterns and investigate the association with cardiometabolic risk. Sedentary time was objectively assessed using a device that accurately distinguishes between sitting, standing and stepping. It was hypothesised that participants who engage in more sedentary time and a lower number of breaks from sedentary time would have increased cardiometabolic risk markers.

3. Methods

3.1. Study design

A cross-sectional design study was used with data collected from participants located in Bedfordshire, UK, at one single time point. Measurements were taken in spring time 2017 (March-June).

3.2 Participants

Participants were 118, 11-12-year-old children (male = 51, female = 67) recruited on a voluntary basis in four schools across Bedfordshire, UK. Informed parental consent and verbal participant assent was obtained prior to any testing procedures following a written explanation of the nature of the research, any associated risks and parents and participants were given the opportunity to ask the research team any questions (8.1: Parent Information Letter and Consent Form). Verbal assent was obtained following a standardised brief explanation of the measurements being taken. Participants completed a pre-study health questionnaire and blood screening questionnaire (8.1: Parent Information Letter and Consent Form), with a signed consent form, which was returned to the research team prior to involvement in the study. Participants were aware that they had the right to withdraw from the study at any point for any reason. Participants were excluded from the study due to the following reasons: had any known blood borne disease, had clinically diagnosed diabetes, were taking glucose-lowering and/or lipid-lowering medication, smoking, had medically diagnosed hypertension, major illness/injury, or other health issues that were deemed as impacting on the associations being explored in the study.

3.3 Ethical approval

Ethical approval was obtained from the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee prior to commencing the study.

3.4 Recruitment

3.4.1 Contact with schools

Seventeen middle schools within Bedford Borough and surrounding areas were contacted by the research team via telephone enquiring about the appropriate person to contact regarding the schools' willingness and availability to help facilitate the study. The suggested person was then contacted via email (8.2: Email to schools). Follow up emails and telephone calls were made if no response was given by the school after one week. This continued until schools had provided an answer of interest or no interest. It was made clear that students that participated in the study and returned their activity monitors on completion of their trial were offered a £5 shopping gift voucher and that a report of the findings would be presented to the school. Four state schools with mixed gender students agreed to take part in the study. Participants were recruited from year 7 for the study.

According to the school's latest Ofsted report, school 1 was smaller than the average school of its type and the majority of students were from White British backgrounds (Ofsted, 2018). The number of students eligible for pupil premium funding was below the national average and the number of students supported by school action plus or with a statement of special educational needs was well below average (Ofsted, 2018). School 2 was a smaller than average school of its type where most students were from White British backgrounds, however the proportion of students from minority

ethnic backgrounds was above average and students who speak English as an additional language was well above average (Ofsted, 2018). The proportion of students eligible for pupil premium is average and the proportion of students with special educational needs or disability is slightly lower than average (Ofsted, 2018). School 3 was an average sized school of its type where most students were from White British backgrounds, however the proportion of students from minority ethnic groups was broadly average (Ofsted, 2018). The proportion of pupils with special educational needs and/or disabilities was above average (Ofsted, 2018). School 4 was a smaller than average school of its type where students were mainly from White British backgrounds (Ofsted, 2018). The proportion of students who speak English as an additional language was broadly average, the proportion of students supported through school action plus or through a statement of special educational needs was well below average and the proportion of students eligible for pupil premium funding was below average (Ofsted, 2018).

3.4.2 Presentation to children

A short presentation during class time or an assembly was given by the research team to children who were potentially eligible for the study (8.3: School Assembly PowerPoint). This was informative and provided an opportunity for them to ask questions and for the researchers to distribute information letters, health screening questionnaires and consent forms for the children to take home to their parents/guardians. It was made clear to potential participants that to be involved in the study, they must agree to all the measures, including the blood sample. Following this, schools were asked to send reminders to parents to complete and return the forms via their text message or email system.

3.4.3 Information and screening forms

Children's parents or guardians provided written informed consent for their child to take part in the study and completed a health screening questionnaire. The forms were handed in to teachers at the school and collected by the research team who screened the volunteers for their eligibility to participate in the study. If information was missing on the forms, this was highlighted and retuned in an envelope to the student to be completed. These forms were collected prior to data collection. On the day of testing, verbal assent was obtained from each child following a verbal explanation of the procedures prior to any testing taking place.

3.5 Procedures

Data collection took place in a room allocated at the relevant school between 7 and 10 am. Participants were instructed to wear loose fitting clothing, such as their PE kit, and to adhere to the following instructions that were provided in writing at least two days prior to testing:

- Fast from 9 pm the night before your testing date (no food or drink apart from small sips of water).
- Avoid exercise for 2 days before the day of testing.
- Tell the research team if you are not feeling well or have been unwell prior to testing.
- Bring a snack to eat for breakfast once measurements have been taken.

3.5.1 Measurement morning

Participants were allocated a 30 minute slot for their measurements to be taken prior to the morning which was communicated by reminder letter (8.4: Reminder Letter) one week before and via a text

message, or email if a number was not provided, the evening before (8.5: Reminder Text Messages). Tanner questionnaires (8.7: Biological Maturity Questionnaire) were also sent with the reminder letters, which were to be returned on the measurement morning.

On arrival participants were signed in, their consent forms checked and Tanner questionnaire handed in. If the child had forgotten their Tanner Questionnaire, and the child was willing to complete the questionnaire, a new form and private space to complete it was provided. Each participant was provided a sticky label with their participant number on to wear throughout the measurement morning and a blank data collection sheet with their corresponding participant number on. The data collection sheet was given to the researcher in their first zone and from then the data collection sheets were swapped between researchers on the different stations as participants moved around.

There were three zones to which participants would visit; a blood sample and activPAL fitting zone, a blood pressure and breakfast questionnaire zone (breakfast questionnaire data not presented in this thesis), and an anthropometrics zone (Figure 1). Participants moved around in groups of 3 – 4 between the measurement zones and the waiting area and were rotated in groups around the stations until all measures had been taken. On completion of all stations, participants were allowed to sit and eat their breakfast snack and have a drink in a separate allocated area before returning to their classroom.

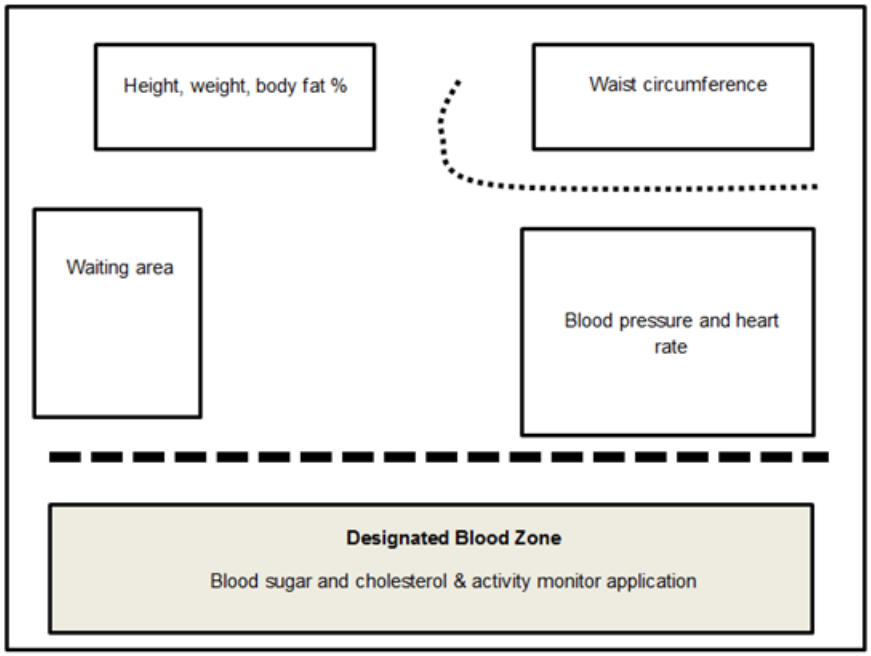

Figure 1. Floor plan of the Measurement Morning Zones

3.6 Measurements

3.6.1 Anthropometrics

3.6.1.1 Standing height

Standing height was measured to the nearest 0.1 cm using a transportable stadiometer (Seca, Hamburg, Germany). Participants were instructed to remove their shoes, stand with their heels together at the base of the stadiometer with their heels, buttocks and head touching the vertical backboard of the stadiometer. Participants were instructed to look straight ahead keeping their head upright (in the Frankfort Plane), to stand as tall as possible and take a deep breath prior to measurement, to ensure the spine was straight and the measurement consistent. The slide was lowered until it reached the top of the skull and the measurement recorded. This was repeated twice and the highest value used.

3.6.1.2 Body mass and body fat

Body mass was measured to the nearest 0.1 kg and body fat% measured by bioelectrical impedance analysis to the nearest 0.1% using the Tanita BC41MA Segmental Body Composition Scales (Tanita Corp., Tokyo, Japan), and has been validated for assessing whole body fat in children (Luque *et al.*, 2014). Participants were instructed to remove their shoes and socks/tights and jumpers for these measures. Body mass was initially measured. A clothing weight of 1.0 kg was entered for all participants during measurement of body fat%. A standard body type was entered for all participants as they were under 18 years old and their sex, age and height inputted. When instructed to do so, participants stood barefoot on metal plates ensuring the heel of their foot was on the back electrode and ball of their foot was on the front electrode. Participants were asked to stand still and look straight ahead. Participants were instructed to pick up the hand grip electrodes and hold them at waist height slightly away from their body, maintaining the still posture as before. Using a safe electrical current, the machine calculated the body content of fat and fat-free mass of the whole body and each limb, based upon the resistance detected in the electrical current. When the measurement was completed a printout was produced and information recorded.

3.6.1.3 Body Mass Index

From height and weight measurements BMI was calculated as: BMI = kg/m^2. BMI-z score was calculated using UK reference values (Cole *et al.*, 1995).

3.6.1.4 Waist circumference

Waist circumference was measured using an anatomical tape measure (HaB Direct, Southam, UK) to the nearest 0.1 cm at the level of the umbilicus (Bailey *et al.*, 2016). The tape was placed around the

trunk in a horizontal plane and the researcher applied sufficient tension to the tape to maintain its position without causing indentation of the skin surface. Once the tape was in place, participants were asked to stand up straight, relax both arms and breathe normally. The measurement was taken on exhalation. Two measures were taken and an average recorded. Abdominal obesity was determined as WC ≥ 90th percentile for age and sex (McCarthy *et al.*, 2001).

3.6.2 Resting heart rate and blood pressure

Resting blood pressure (BP) and heart rate (HR) was measured using an Omron M5-I automatic blood pressure monitor (Omron Matsusaka Co Ltd., Matsusaka, Japan). The participant sat and rested for 5 minutes prior to measurement. The cuff of the monitor was fitted fairly loosely around the bare left arm of the participant with the green band on the cuff 1 – 2 cm above the elbow crease on the inside of the arm. The arm rested on a table with the hand in a supinated position and the cuff level with the heart. It was explained to the participant that the cuff would inflate and feel tight around the arm for a few seconds. Two BP readings were taken, with a 2-minute interval between each where the cuff was loosened, and an average of the two readings was recorded in line with previous research to allow for device variability (Bailey *et al.*, 2012a). Elevated SBP and/or DBP was defined as SBP and/or DBP ≥ 90th percentile for the child's age and sex (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

3.6.3 Full lipid profile and blood glucose

Blood samples were obtained by finger prick with an auto lancet followed by gentle massage to promote the appearance of small drops of blood at the skin's surface. The first drop of blood was discarded and then 100 µl blood was collected into a capillary tube which was then transferred into a

cassette sample well for the measurement of lipid profile and glucose. The cassette was placed in the drawer of a Cholestech LDX® analyzer (Alere San Diego Inc., California, USA) to obtain results within approximately 6 minutes. If insufficient blood was obtained, a second finger was pricked if the child was willing and not in any way distressed by the first. This system has been validated in adults (Parikh *et al.*, 2009) and has been used in previous paediatric research (Ahrens *et al.*, 2014; Bailey *et al.*, 2016).

Impaired fasting glucose was defined as ≥ 5.6 mmol · L^{-1} (Zimmet *et al.*, 2007). Hypercholesterolemia was defined as ≥ 5.17 mmol · L^{-1} (National Cholesterol Education Program Expert Panel on Blood Cholesterol Levels in Children and Adolescents, 1992). Triglycerides were considered high between 1.02 – 1.46 mmol · L^{-1} (90 – 129 mg/dL) (National Cholesterol Education Program Expert Panel on Blood Cholesterol Levels in Children and Adolescents, 1992). The present study defined hypertriglyceridemia ≥ 1.24 mmol · L^{-1} as this is the mid-point between the range 1.02 – 1.46 mmol · L^{-1} (Cook *et al.*, 2003; Bailey *et al.*, 2016). HDL in children was considered low between 0.91 – 1.16 mmol · L^{-1} (35 – 45 mg/dL) (National Cholesterol Education Program Expert Panel on Blood Cholesterol Levels in Children and Adolescents, 1992). The present study defined low HDL as ≤ 1.03 mmol · L^{-1} as this is the mid-point between the range 0.91 – 1.16 mmol · L^{-1} (Cook *et al.*, 2003; Bailey *et al.*, 2016).

3.6.4 Clustered cardiometabolic risk score

Clustered cardiometabolic risk score (sex specific) was calculated by summing the z scores for WC, DBP, TC:HDL ratio, triglycerides and glucose (Bailey *et al.*, 2016). A non-obesity clustered cardiometabolic risk score was calculated by adding the z scores for DBP, TC:HDL ratio, triglycerides and glucose (Ekelund *et al.*, 2007). Increased clustered cardiometabolic risk score (3.08) and increased non-obesity clustered cardiometabolic risk score (2.58) were defined as ≥ 1 SD in risk score above the pooled mean (Andersen *et al.*, 2006). These scores were calculated as they provide broader insight to

cardiometabolic risk accounting for daily variations and has previously been used in paediatric research (Bailey *et al.*, 2016; Saunders *et al.*, 2013b).

3.6.5 Metabolic Syndrome

MetS was defined as ≥ 3 of the following cardiometabolic risk factors: high WC, low HDL, hypertriglyceridaemia, high SBP or DBP and/or impaired fasting glucose (Bailey *et al.*, 2016).

3.6.6 Indices of Multiple Deprivation Scores

The post code of the children's home address was reported on the consent form returned to researchers. The post codes were entered into the website www.imd-by-postcode.opendatacommunities.org (Department for Communities and Local Government, 2015) and an indices of multiple deprivation (IMD) score calculated.

3.6.7 Sedentary behaviour and physical activity

3.6.7.1 activPAL

The activPAL device (PAL technologies, Glasgow, Scotland) measures 53 x 35 x 7mm, weighs approximately 20 g and using the inclination on the thigh can identify postural changes. The activPAL was attached to the participant during the above testing procedures. Before attaching to the participant, the activPAL device was covered in a nitrile flexible sleeve to protect the device from water. The device was fitted to the right thigh of the participant half way between the crease of the hip joint and the knee, in accordance with manufacturer instructions, with a 10 x 10 cm hypo-allergenic transparent film roll (Tegaderm, Neuss, Germany; Hypafix, BSNmedical, UK). Extra micropore tape was placed around the edge of the taped device if required. The device was set up to start recording at 12 midnight the day following testing and recorded data in 15 s epochs (time intervals) for the next seven

consecutive days. Data was recorded continuously including sleep and instances where the device could get wet, unlike previous studies that used accelerometers (Bailey *et al.*, 2016; Colley *et al.*, 2013) whereby participants were required to remove the devices for such instances. In the present study participants were required to remove the activPAL device if swimming to prevent the device being lost. This enabled a better representation of behaviours across the entire data collection period.

Participants were instructed to wear the device continuously throughout these seven days, which enhanced wear time compliance (Tudor-Locke *et al.*, 2015). Participants were advised to avoid touching the device during the data collection period as it may have become unstuck and/or provided inaccurate readings. Participants or their parents (dependant on the parent's preference) received a daily text message to a mobile telephone number that parents provided on the consent form, from the research team to remind the participants to wear their activPAL device. Participants were provided additional attachment dressings in case this needed to be replaced during the measurement period. Parents, participants and school teachers were provided written instructions on how to re-attach the device.

3.6.7.2 Data reduction

Total sedentary time per day, time spent standing per day and time spend in light physical activity was calculated. A prolonged sedentary bout in this study was defined as a period of sedentary time ≥ 30 minutes in a sitting/reclining posture with no tolerance time within sedentary bouts (Altenburg *et al.*, 2015). Time spent in prolonged sedentary bouts was reported as minutes/day. A break in sedentary time was defined in the present study as a non-sedentary period in between two sedentary bouts (Altenburg and Chinapaw, 2015), such as a sit to stand transition. The number of breaks in sedentary

time was reported. All variables were calculated for each day and then averaged across the number of days included.

The activPAL gives a direct estimate of body inclination and does not rely on thresholds or cut points, and thus allows time spent standing to be estimated independently and can detect changes in sitting/lying time (Harrington *et al.*, 2011). Time spent sitting/lying can produce counts, for instance when fidgeting or gentle movements whilst sleeping, and non-wear time is reflected by extended periods of continuous zero counts. Periods and patterns of sitting per day (total sitting time, prolonged sitting bouts [bouts ≥ 30 minutes] and breaks in sitting time), standing, stepping time, and time in different physical activity intensities (light and MVPA) were determined using an algorithm developed (Winkler *et al.*, 2016) for use with Stata data analysis and statistical software (Stata, Texas, US). Light PA is defined as stepping activity < 3 METs and MVPA is defined as stepping activity > 3 METs within the algorithm (Winkler *et al.*, 2016). It is important to note that although the use of activPAL devices has been validated for use in children (Sellers *et al.*, 2016; Aminian and Hinckson, 2012; Ridley *et al.*, 2016), the algorithm is only currently validated in adults (Winkler *et al.*, 2016). To date, no activPAL algorithm has been validated in children. Inclusion criteria for valid wear time was a minimum of four days, including at least one weekend day, and a minimum of 10 hours of wear time was required for a day to be valid (Harrington *et al.*, 2011; Winkler *et al.*, 2016).

3.6.7.3 Daily log

Participants were given a log sheet in which they completed sections on a daily basis (8.6: Activity log). The sheet contained instructions on how to fill in the daily log, an example of a day filled in, guidance on how to re-attach the activPAL and eight blank day templates to complete. Each day they recorded their sleep (what time they woke up, got out of bed, went to bed, went to sleep) and information

about their activPAL device (removal of the monitor with reasoning and timings). Participants were advised not to remove the monitor; however, information was provided in the booklet on how to reattach it if required. Participants were also required to record whether or not they consumed breakfast each day (data not presented in this thesis). This log sheet was given to participants when their activPAL was attached and collected by researchers when they collected the activPAL devices at the end of the data collection period. If a participant forgot their log on the day of collection they returned it the following day to their teacher who liaised with the research team to collect it. The school was provided with spare log sheets in case a participant misplaced their original one.

3.6.8 Biological maturity

Biological maturity data was obtained from children via self-assessment using the Tanner scale (Tanner, 1962) for genitalia/breast and pubic hair development (8.7: Biological Maturity Questionnaire). The maturity documents in the appendices outline the process for participants to provide this data. After informed consent was obtained, each participant was provided the relevant (boy or girl) maturity rating document in a closed envelope to take home and show to their parent and complete in their own time. The children were asked to return the coded self-assessment response sheet in a sealed envelope that was provided to a named school staff member or a member of the research team on the following day. Children who did not return their forms were followed up via the research team contacting the parents on the contact number provided. Tanner questionnaire envelopes were opened and data entered into an Excel spreadsheet by a researcher that was not connected with the present study.

3.7 Statistical analysis

IBM SPSS Statistics Processor v23 for Windows (IBM Corporation., New York) was used for all statistical analysis. Skewness and kurtosis were analysed, in addition to visual checks of Q-Q plots, and variables that were not normally distributed were log10 transformed (weight, BMI, waist circumference and TC: HDL ratio) in line with previous similar studies (Bailey *et al.*, 2016; Ekelund *et al.*, 2007). Scatter plots were assessed for linearity and homoscedasticity and deemed that the data met the assumptions to run multiple regression analyses. Data was checked for multicollinearity and identified a Variance Inflation Factor (VIF) score > 10 for both weartime and total sedentary time variables. The regression analysis automatically removed total sedentary time from the models, thus results for total sedentary time were limited to partial correlation analysis only. Descriptive data is presented as mean ± SD. Sex, IMD scores, biological maturity, school attended, and activPAL weartime were significantly corelated with ≥1 cardiometabolic risk factor and thus were adjusted for in the analyses (Altenburg *et al.*, 2015). Partial correlation and multiple regression analysis was used to assess associations between cardiometabolic risk marker levels (TC, HDL, LDL, triglycerides, non-HDL, TC/HDL, glucose, body fat%, WC, BMI, resting HR, BP, and cardiometabolic risk scores) and total sedentary time, standing time, light physical activity, number of breaks in sedentary time per day, total daily time spent in prolonged sedentary bouts (≥ 30 minutes) and the number of prolonged sedentary bouts per day. Partial correlations were used to ascertain individual relationships between each independent and dependent variable, whilst eliminating the effect of confounding variables. Multiple regression was used to ascertain the ability of each independent variable to predict each of the dependent variables whilst adjusting for confounding variables. Additional analyses were conducted additionally adjusting for MVPA as this has previously impacted upon the association of sedentary behaviour with cardiometabolic risk markers (Chaput *et al.*, 2013). The strength of correlations were interpreted as follows: .00 to .30 = negligible; .30 to .50 = low/weak positive/negative; .50 to .70 = moderate

positive/negative; .70 to .90 high/strong positive/negative; .90 to 1 very high/strong positive/negative (Mukaka, 2012). The level of significance was accepted at $p \leq 0.05$ to reduce the likelihood of a type I error in line with previous related research (Ekelund *et al.*, 2007; Buchan *et al.*, 2014).

4. Results

4.1 Descriptive Characteristics

Of the 610 information sheets distributed, 148 participants returned consent forms, of which 20 participants withdrew from the study prior to data collection. Ten participants did not provide valid activPAL data (six did not meet weartime criteria, two devices malfunctioned, and two devices were not returned) and thus were excluded from the analysis. A total of 118 participants (67 girls) were included in the present analysis for all measures. Three participants (two girls) withdrew from the blood sampling during the measurement morning and 115 participants were thus included for analysis of blood markers.

The cardiometabolic risk marker descriptive characteristics of the participants are summarised in Table 1, sedentary behaviour and physical activity descriptive characteristics are summarised in Table 2 and descriptive characteristics of IMD scores are summarised in Table 3. The prevalence of abdominal obesity in the whole sample was 37.3% (n = 44) (35.3% boys [n = 18] and 38.8% of girls [n = 26]), elevated SBP 2.6% (n = 3), and elevated DBP 3.4% (n = 4). From the 115 participants that provided blood samples, the prevalence of hypercholesterolemia was 41.7% (n = 48), hypertriglyceridemia 28.7% (n = 33), low HDL 4.4% (n = 5), impaired fasting glucose 6.1% (n = 7), MetS was prevalent in 4.4% of participants (n = 5), while 13.9% had an increased clustered cardiometabolic risk score (n = 16), and 8.7% had an increased non-obesity clustered cardiometabolic risk score (n = 10).

Table 1. Descriptive cardiometabolic risk marker characteristics for participants

	All (n = 118)	Boys (n = 51)	Girls (n = 67)
Height (cm)	154.3 ± 7.2	153.4 ± 6.6	154.9 ± 7.5
Weight (kg)	45.3 ± 11.3	43.7 ± 10.4	46.5 ± 11.9
Body Mass Index (kg/m^2)	18.9 ± 3.9	18.5 ± 3.5	19.3 ± 4.4
Body Mass Index z score	0.01 ± 1.02	-0.12 ± 0.89	0.10 ± 1.10
Body fat%	23.3 ± 7.1	21.3 ± 6.9	24.9 ± 6.9
Waist Circumference (cm)	67.3 ± 10.1	67.6 ± 9.0	67.0 ± 10.9
Systolic Blood Pressure (mmHg)	104 ± 10.93	101 ± 11.17	107 ± 10.18
Diastolic Blood Pressure (mmHg)	67 ± 7.62	65 ± 7.41	68 ± 7.64
Heart Rate (bpm)	79 ± 11.59	75 ± 10.86	82 ± 11.33
TC ($mmol \cdot L^{-1}$)	5.94 ± 2.79	5.76 ± 2.65	6.08 ± 2.90
HDL ($mmol \cdot L^{-1}$)	2.12 ± 1.08	2.18 ± 1.08	2.07 ± 1.08
Triglycerides ($mmol \cdot L^{-1}$)	1.50± 1.53	1.26 ± 1.30	1.68 ± 1.67
LDL ($mmol \cdot L^{-1}$)	3.36 ± 1.76	3.21 ± 1.61	3.47 ± 1.87
Non-HDL ($mmol \cdot L^{-1}$)	3.82 ± 1.93	3.59 ± 1.74	3.99 ± 2.05
TC:HDL ratio	2.93 ± 0.80	2.72 ± 0.46	3.09 ± 0.95
TRG:HDL ratio	1.55 ± 1.36	1.13 ± 0.87	1.88 ± 1.57
Glucose ($mmol \cdot L^{-1}$)	4.96 ± 0.49	4.93 ± 0.43	4.98 ± 0.53
Clustered risk score	0.01 ± 3.08	-0.54 ± 2.46	0.44 ± 3.44
Non-obesity clustered risk score	0.56 ± 2.58	-0.59 ± 2.03	0.55 ± 2.85

Data presented as mean ± SD.

TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table 2. Descriptive sedentary behaviour and physical activity characteristics for participants

	All (n = 118)	Boys (n = 51)	Girls (n = 67)
activPAL weartime (minutes/day)	849.91 ± 42.6	852.00 ± 42.6	848.40 ± 42.6
Total sedentary time (minutes/day)	522.60 ± 67	513.60 ± 1.13	529.20 ± 52.3
Standing time (minutes/day)	177.00 ± 39.6	165.60 ± 45.6	185.40 ± 31.8
Light physical activity (minutes/day)	60.60 ± 15	69.60 ± 13.2	54.00 ± 12.6
MVPA (minutes/day)	90.00 ± 24	103.20 ± 22.2	79.80 ± 19.8
MVPA (% meeting 60min/day guidelines)	91.53 ± 0.40	98.31 ± 0.37	93.22 ± 0.33
Number of breaks in sedentary time per day	81.32 ± 11.50	82.71 ± 17.66	80.26 ± 19.17
Number of prolonged sedentary bouts (≥ 30 minutes)	3.7 ± 1.2	3.6 ± 1.4	3.8 ± 1.1
Time spent in prolonged sedentary bouts (minutes/day)	265.91 ± 93	262.63 ± 107.4	268.42 ± 81.6

Data presented as mean ± SD.

MVPA, moderate-to-vigorous physical activity

Table 3 Descriptive characteristics for Indices of Multiple Deprivation scores

	IMD Scores	IMD Quintile for England	Percentage of total sample
School 1	10.65 ± 7.51	1.70 ± 1.08	16.9% (n = 20)
School 2	19.63 ± 8.55	3.15 ± 0.95	22.9% (n = 27)
School 3	10.53 ± 7.07	1.93 ±1.03	43.3% (n = 51)
School 4	12.54 ± 9.38	1.95 ± 1.22	16.9% (n = 20)
All	13.07 ± 8.68	2.19 ± 1.18	100%

Data presented as mean ± SD.
IMD – Indices of Multiple Deprivation
IMD Quintile – Q1 ≤ 8.49 (least deprived); Q2 8.5 – 13.79; Q3 13.8 – 21.35; Q4 21.36 – 34.17; Q5 ≥ 34.18 (most deprived).

4.2 Sedentary behaviour and cardiometabolic risk

4.2.1 Correlation analysis

Partial correlation data adjusting for sex, IMD score, school, Tanner stage and weartime is reported in **Error! Reference source not found.**4. Total sedentary time was significantly positively correlated with glucose but not significantly correlated with any other risk marker. No significant correlations were found between standing time and any risk marker. Light physical activity was significantly negatively correlated with glucose, body fat% and clustered risk score, however, all other risk markers were non-significantly correlated with this variable. The number of breaks in sedentary time was significantly negatively correlated with body fat% and weight and non-significantly correlated with all other markers. The number of prolonged sedentary bouts was significantly positively correlated with glucose and non-significantly correlated with all other risk markers. The time spent in prolonged sedentary bouts was significantly positively correlated with glucose; no significant correlations were found with any other risk marker.

Error! Reference source not found.5 shows partial correlation data that additionally adjusted for MVPA. Total sedentary time became non-significantly correlated with glucose and remained uncorrelated with all other risk markers. Standing time continued to show no significant correlation with any risk marker. The correlation between light physical activity and glucose and clustered risk score became non-significant when MVPA was adjusted for, however, DBP became significantly negatively correlated. Body fat% remained significantly negatively correlated with light physical activity and all other risk markers remained non-significantly correlated. The number of breaks in sedentary time remained significantly negatively correlated with body fat% and weight and non-significantly correlated with all other markers. The number of prolonged sedentary bouts became non-significantly correlated with glucose when MVPA was additionally adjusted for and all other markers remained non-

significantly correlated. The time spent in prolonged sedentary bouts became non-significantly correlated with glucose when MVPA was additionally adjusted for; no significant correlations were found with any other risk marker.

Table 4. Partial correlations between sedentary behaviour variables, light physical activity and cardiometabolic risk markers in 11-12-year-old children, excluding MVPA

	Total sedentary time (minutes/ day)	Standing time (minutes/ day)	Light physical activity (minutes/ day)	Number of breaks in sedentary time per day	Number of prolonged sedentary bouts per day	Total time spent in prolonged sedentary bouts (minutes/ day)
Weight[a] (kg)	.138	-.101	-.148	-.238*	.082	.082
BMI[a] (kg/m^2)	.075	-.025	-.116	-.163	.016	.003
WC[a] (cm)	.172	-.139	-.116	-.179	.081	.099
Body Fat%	.181	-.124	-.282**	-.276**	.116	.114
Systolic Blood Pressure (mmHg)	-.070	.118	-.073	-.067	.057	.014
Diastolic Blood Pressure (mmHg)	-.002	-.005	-.125	-.132	.108	.079
TC (mmol · L^{-1})	.027	.032	-.103	.015	.103	.111
HDL (mmol · L^{-1})	-.034	.113	-.103	.055	.040	.048
Triglycerides (mmol · L^{-1})	.039	.037	-.079	.041	.028	.034
LDL (mmol · L^{-1})	.050	-.023	-.079	-.008	.124	.127
Non-HDL (mmol · L^{-1})	.058	-.015	-.091	-.003	.120	.127
TC: HDL[a]	.074	-.094	.001	-.064	.064	.069
TRG: HDL	.112	-.086	-.080	-.034	.078	.084
Glucose (mmol · L^{-1})	.265**	-.147	-.290**	-.099	.220**	.269**
Clustered risk score	.180	-.115	-.200*	-.142	.165	.181
Non-obesity clustered risk score	.150	-.085	-.196	-.102	.167	.180

[a] log-transformed Significant associations * $p \leq 0.05$ ** $p \leq 0.01$

Partial correlations adjusted for sex, IMD score, school and Tanner stage and weartime.

BMI, Body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table 5. Partial correlations between sedentary behaviour variables, light physical activity and cardiometabolic risk markers in 11-12-year-old children, adjusting for MVPA

	Total sedentary time (minutes/ day)	Standing time (minutes/ day)	Light physical activity (minutes/ day)	Number of breaks in sedentary time per day	Number of prolonged sedentary bouts per day	Total time spent in prolonged sedentary bouts (minutes/ day)
Weight[a] (kg)	.094	-.072	-.111	-.222*	.038	.036
BMI[a] (kg/m^2)	.016	.006	-.080	-.147	-.032	-.048
WC[a] (cm)	.098	-.097	-.045	-.152	.014	.031
Body Fat%	.161	-.097	-.274**	-.262**	.080	.077
Systolic Blood Pressure (mmHg)	-.081	.119	-.098	-.072	.075	.026
Diastolic Blood Pressure (mmHg)	.091	-.039	-.209*	-.157	.174	.145
TC (mmol · L^{-1})	-.028	.058	-.080	.031	.082	.090
HDL (mmol · L^{-1})	-.096	.137	-.091	.068	.019	.028
Triglycerides (mmol · L^{-1})	-.073	.085	-.013	.072	-.037	-.033
LDL (mmol · L^{-1})	.027	-.009	-.066	.002	.118	.122
Non-HDL (mmol · L^{-1})	.009	.010	-.061	.014	.097	.105
TC: HDL[a]	.073	-.089	.022	-.058	.055	.061
TRG: HDL	.057	-.055	-.031	-.012	.034	.039
Glucose (mmol · L^{-1})	.102[b]	-.060	-.174 [b]	-.040	.105 [b]	.158 [b]
Clustered risk score	.097	-.067	-.138 [b]	-.111	.103	.119
Non-obesity clustered risk score	.078	-.043	-.148	-.074	.118	.131

[a] log-transformed [b] Different from Table 3 Significant associations * $p \leq 0.05$ ** $p \leq 0.01$

Partial correlations adjusted for sex, IMD score, school and Tanner stage, total sedentary time, weartime and MVPA.

BMI, Body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

4.2.2 Multiple linear regression analysis

Variables entered into the regression models included total sedentary time, standing time, time spent in light physical activity, number of breaks in sedentary behaviour and prolonged bouts of sedentary behaviour and covariates of sex, IMD score, tanner score, school, weartime (model 1). In Model 2, these covariates were entered in addition to MVPA. Due to high collinearity with weartime, total sedentary time was removed from the analysis in both models.

4.2.2.1 Standing time

In regression model 1 (Error! Reference source not found.6), standing time was significantly negatively associated with weight and body fat% and significantly positively associated with HDL. In regression model 2 (Table 7), standing time remained significantly negatively associated with weight and body fat% and significantly positively associated with HDL. In addition, WC became significantly negatively associated with standing time in model 2. In both regression models, no significant association was found between standing and BMI, SBP, DBP, HR, TC, triglycerides, LDL, non-HDL, TC: HDL, TRG: HDL, glucose, clustered risk score or non-obesity clustered risk score.

4.2.2.2 Light physical activity

Light physical activity was significantly negatively associated with body fat% in regression model 1, however this association was weakened when MVPA was additionally adjusted for in regression model 2 and became non-significant. No significant association was found between light physical activity and any other risk marker in both models.

4.2.2.3 Breaks in sedentary time

In both regression models the number of breaks in sedentary time per day was significantly negatively associated with weight, BMI, WC and body fat% and significantly positively associated with TC and HDL. In regression model 1, HR was significantly positively associated with the number of breaks in sedentary time per day, however this association was attenuated in model 2 when additionally adjusting for MVPA and became non-significant. In both models, no association was found between SBP, DBP, triglycerides, LDL, non-HDL, TC: HDL, TRG: HDL, glucose, clustered risk score and non-obesity clustered risk score.

4.2.2.4 Time in prolonged sedentary bouts

Total time spent in prolonged sedentary bouts was significantly negatively associated with weight, BMI, WC and body fat% and significantly positively associated with TC and HDL in both regression models. In regression model 1, LDL and non-HDL were significantly positively associated with total time spent in prolonged sedentary bouts, however, this was attenuated in regression model 2 and became non-significant.

Table 6. Adjusted regression analysis between sedentary behaviour variables, light physical activity and cardiometabolic risk markers in 11-12-year-old children, excluding MVPA (Model 1)

	Standing time (minutes/ day)	Light physical activity (minutes/ day)	Number of breaks in sedentary time per day	Total time in prolonged sedentary bouts (minutes/day)
Weight[a] (kg)	-.253 (-.074, -.001)*	-.141 (-.156, .049)	-.591 (-.005, -.002)***	-.590 (-.057, -.016)***
BMI[a] (kg/m^2)	-.150 (-.049, .013)	-.149 (-.133, .041)	-.526 (-.004, -.001)***	-.581 (-.047, -.012)***
WC[a] (cm)	-.252 (-.048, .001)	-.084 (-.089, .050)	-.514 (-.003, -.001)**	-.473 (-.032, -.004)*
Body Fat%	-.274 (-5.615, -.260)*	-.310 (-16.012, -.934)*	-.497 (-.299, -.075)***	-.624 (-4.300, -1.265)***
Systolic Blood Pressure (mmHg)	.097 (-2.742, 6.013)	-.098 (-16.550, 8.100)	-.177 (-.288, .077)	-.151 (-3.534, 1.411)
Diastolic Blood Pressure (mmHg)	-.056 (-2.603, 3.932)	-.021 (-9.839, 8.563)	-.070 (-.165, .108)	.037 (-1.664, 2.027)
Heart Rate (bpm)	-.023 (-4.925, 4.113)	-.265 (-24.694, .754)	.333 (.018, .396)*	.153 (-1.429, 3.676)
TC (mmol · L^{-1})	.241 (-.023, 2.041)	-.051 (-3.538, 2.428)	.343 (.007, .095)*	.421 (.126, 1.348)*
HDL (mmol · L^{-1})	.309 (.115, .898)*	-.152 (-1.786, .479)	.404 (.007, .040)**	.417 (.054, .518)*
Triglycerides (mmol · L^{-1})	.151 (-.290, 1.002)	-.145 (-2.760, .974)	.186 (-.012, .043)	.134 (-.250, .515)
LDL (mmol · L^{-1})	.162 (-.249, 1.103)	.032 (-1.733, 2.175)	.264 (-.004, .054)	.374 (.011, .811)*
Non-HDL (mmol · L^{-1})	.178 (-.235, 1.263)	.005 (-2.128, 2.201)	.272 (-.004, .060)	.376 (.011, .898)*
TC: HDL[a]	-.119 (-.064, .026)	.157 (-.063, .196)	-.145 (-.003, .001)	-.021 (-.028, .025)
TRG: HDL	-.070 (-.737, .443)	-.044 (-1.947, 1.461)	-.026 (-.027, .023)	-.035 (-.380, .318)
Glucose (mmol · L^{-1})	.163 (-.077, .320)	-.228 (-1.022, .126)	.148 (-.005, .012)	.339 (-.012, .223)
Clustered risk score	-.002 (-1.341, 1.321)	-.105 (-5.145, 2.551)	-.130 (-.079, .035)	.003 (-.783, .793)
Non-obesity clustered risk score	.078 (-.801, 1.418)	-.100 (-4.244, 2.170)	.008 (-.046, .049)	.137 (-.430, .883)

Standardised beta values from multiple regressions. Data are standardised regression coefficients (95% CI). All outcomes are unadjusted.
[a] log-transformed Significant associations *$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

Table 7. Adjusted regression analysis between sedentary behaviour variables, light physical activity and cardiometabolic risk markers in 11-12-year-old children, including MVPA (Model 2)

	Standing time (minutes/ day)	Light physical activity (minutes/ day)	Number of breaks in sedentary time per day	Total time in prolonged sedentary bouts (minutes/ day)
Weight[a] (kg)	-.270 (-.076, -.004)*	-.013 (-.118, .109)	-.661 (-.005, -.002)***	-.678 (-.063, -.021)***
BMI[a] (kg/m^2)	-.168 (-.051, .010)	-.012 (-.100, .093)	-.601 (-.004, -.001)***	-.675 (-.052, -.016)***
WC[a] (cm)	-.272 (-.049, -.001)* [b]	.069 (-.060, .093)	-.597 (-.003, -.001)***	-.577 (-.036, -.008)***
Body Fat%	-.286 (-5.741, -.388)*	-.220 (-14.447, 2.424) [b]	-.546 (-.321, -0.90) ***	-.685 (-4.629, -1.495)***
Systolic Blood Pressure (mmHg)	.090 (-2.882, 5.921)	-.046 (-15.855, 11.887)	-.206 (-.312, .067)	-.186 (-3.889, 1.265)
Diastolic Blood Pressure (mmHg)	.072 (-2.406, 4.104)	-.140 (-14.476, 6.041)	-.005 (-.142, .138)	.118 (-1.325, 2.487)
Heart Rate (bpm)	-.028 (-5.040, 4.060)	-.229 (-24.676, 4.002)	.313 (-.001, .391) [b]	.128 (.-1.723, 3.603)
TC (mmol · L^{-1})	.239 (-.045, 2.041)	-.037 (-3.755, 2.947)	.334 (.003, .096)*	.410 (.070, 1.363)*
HDL (mmol · L^{-1})	.312 (.116, .907)*	-.169 (-2.000, .543)	.415 (.007, .042)**	.432 (.051, .542)*
Triglycerides (mmol · L^{-1})	.137 (-.328, .971)	-.067 (-2.499, 1.673)	.134 (-.018, .040)	.069 (-.335, .470)
LDL (mmol · L^{-1})	.160 (-.261, 1.105)	.043 (-1.899, 2.491)	.257 (-.006, .054)	.365 (-.022, .824) [b]
Non-HDL (mmol · L^{-1})	.172 (-.259, 1.254)	.034 (-2.172, 2.688)	.253 (-.008, .060)	.351 (-.044, .893) [b]
TC: HDL[a]	-.129 (-.066, .024)	.210 (-.056, .234)	-.180 (-.003, .001)	-.066 (-.032, .024)
TRG: HDL	-.082 (-.767, .420)	.023 (-1.781, 2.033)	-.395 (-.032, .021)	-.437 (-.449, .287)
Glucose (mmol · L^{-1})	.158 (-.082, .319)	-.203 (-1.041, .247)	.131 (-.005, .012)	.317 (-.025, .223)
Clustered risk score	-.014 (-1.407, 1.275)	-.042 (-4.834, 3.783)	-.172 (-.088, .031)	-.050 (-.930, .732)
Non-obesity clustered risk score	.072 (-.835, 1.406)	-.070 (-4.321, 2.879)	-.013 (-.052, .048)	.111 (-.511, .878)

Standardised beta values from multiple regressions. Data are standardised regression coefficients (95% CI). All outcomes are adjusted for sex, IMD score, school and Tanner stage, total sedentary time, weartime and MVPA.

[a] log-transformed [b] Different from Partially adjusted regression model Significant associations *$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

5. Discussion

The main findings of this study were that breaks in sedentary time were significantly negatively associated with weight, BMI, WC and body fat% and significantly positively associated with HDL and TC. Time in prolonged sedentary bouts was significantly negatively associated with weight, BMI, WC and body fat% and significantly positively associated with HDL and TC. Standing time was significantly negatively associated with weight, WC and body fat% and significantly positively associated with HDL.

5.1 Sample demographics

The descriptive characteristics of the sample used in the present study are similar to those reported in the Health Survey for England (HSE) 2016 (Fuller *et al.*, 2017). In 2016, the average height of an 11-12-year-old was 153.2 cm and 153.4 cm in boys and girls (Fuller *et al.*, 2017), respectively, compared to 153.4 cm and 152.1 cm in the present study. The HSE 2016 reported the average weight for an 11-12-year-old was 46 kg and 46.6 kg for boys and girls (Fuller *et al.*, 2017), respectively, compared to 43.7 kg and 46.5 kg in the present study. The average 11-12-year-old has a BMI score of 21.1 kg/m^2 and 19.9 kg/m^2 for boys and girls (Fuller *et al.*, 2017), respectively, compared to 18.5 kg/m^2 and 19.3 kg/m^2 in the present study. Participants in the present study were highly active with 91.53% (98.31% of boys and 93.22% girls) meeting the recommendations of at least 60 minutes of MVPA per day (World Health Organisation, 2010). According to the HSE 2016 report, only 18% of boys and 13% of girls aged 11-12-years-old met this guideline (Fuller *et al.*, 2017). Due to the sample in the present study being highly active, it is difficult to generalise findings from this study to less active children.

To the authors knowledge there is no published data regarding the other metabolic risk markers and prevalence rates in children in England or the UK. In children aged 9-11 years old in the United States between 2011-2014, the average prevalence of hypercholesterolemia was 7.3% (Nguyen *et al.*, 2015), compared to 41.7% in the present study, and the prevalence of low HDL was 10.3% (Nguyen *et al.*, 2015), compared to 4.4% in the present study. One explanation for the differences in prevalence could be the use of different thresholds to define hypercholesterolemia, which was defined as ≥ 5.17 mmol · L^{-1} in the present study but defined as ≥ 11.1 mmol · L^{-1} by Nguyen *et al.* (2015);- and low HDL defined as ≤ 1.03 mmol · L^{-1} in the present study but defined as < 2.2 mmol · L^{-1} by Nguyen *et al.* (2015). In a systematic review of 85 papers, the prevalence of MetS in children was 3.3% (Friend *et al.*, 2013), similar to that found in the present study of 4.4%.

A large proportion of the participants in this sample had IMD scores in the first or second quintile of IMD scores in England, which although was controlled for in the statistical analysis, may influence the associations found between sedentary behaviour and cardiometabolic risk. Previous research found associations between socioeconomic status (SES) and sedentary behaviours; more lower SES children spent time in sedentary behaviours than high SES children (Fairclough *et al.*, 2009) and lower SES home environments have been shown to provide more opportunities for children to engage in sedentary behaviours and less opportunities for PA compared to higher SES home environments (Tandon *et al.*, 2012). In addition lower SES children have been shown to have higher body mass and BMI than higher SES children (Drenowatz *et al.*, 2010). Future research should consider using populations from specific SES groups and/or a representative sample from each SES group.

5.2 Patterns of sedentary behaviour

5.2.1 Total sedentary time

In this study, boys accumulated 524 minutes (8 hours 44 minutes) and girls 526 minutes (8 hours 46 minutes) of total sedentary time per day. Although to date there are no national statistics regarding sedentary time in children, this amount is similar to that found by Colley *et al.* (2013) in children aged 11 – 14 years old; boys accumulated 508 minutes and girls 524 minutes of total sedentary time per day. Children in the HAPPY study aged 10 – 14 years old accumulated 504 minutes of sedentary time per day (Bailey *et al.*, 2016). This is similar to the results found in the present study and this is likely due to the similar age range and location of residence of the samples. It has been previously reported that children in Europe aged 10 – 14 years old demonstrate approximately 7 – 8 hours of objectively measured sedentary time each day (Bailey *et al.*, 2012a; Verloigne *et al.*, 2013), however, the present study suggests that this is actually between 8 – 9 hours. One reason for this could be that in the 5 years between the previous and present studies, children have become more sedentary. In addition, the use of the activPAL inclinometer may have been more sensitive to detecting sedentary behaviours than previously used accelerometers (Aminian and Hinckson, 2012). Variations regarding the average time children spend in sedentary time is evident in the literature; Carson and Janssen (2011) reported 6 – 19 year olds spent an average of 424 minutes in sedentary time per day, while Janssen *et al.* (2016) found 12 year old children engaged in 467 minutes of sedentary time per day and 8 – 11 year old children demonstrated 365 minutes. These figures are lower than those found in the present study, which may be due to the type of accelerometer used; in the previous studies the accelerometers used were unable to differentiate posture, which thus may have misclassified standing time as sedentary behaviour (Chastin and Granat, 2009). In addition, a wider age range of children were used, including younger children who tend to

exhibit lower levels of sedentary time than older children (Jago *et al.*, 2017; Mann *et al.*, 2017), thus affecting the mean scores presented. Future studies should consider splitting their samples into smaller age categories to permit conclusions around sedentary time and risk markers for specific age groups. In addition, future research should utilise postural allocation and accelerometry when investigating patterns of sedentary behaviour in children to ensure true sedentary behaviours, i.e. is a sitting or lying posture, are measured. The present study highlights that children aged 11-12-years-old engage in 8-9 hours of sedentary time per day, which is higher than previously reported in other studies.

5.2.2. Time in prolonged sedentary bouts

The present study found that children aged 11 – 12 years old spend an average of 265 minutes in prolonged sedentary bouts ≥ 30 minutes per day. This is similar to findings of Bailey *et al.* (2016), who found that children aged 10 – 14 years old spent 260 minutes in prolonged bouts of ≥ 20 minutes, but higher than that found by Carson and Janssen (2011) with 204 minutes in prolonged sedentary bouts ≥ 30 minutes. A potential reason for the differences in prolonged sedentary bouts could be that the present study and Carson and Janssen (2011) defined a prolonged bout as ≥ 30 minutes rather than the ≥ 20 minutes defined by Bailey *et al.* (2016), thus direct comparisons cannot be made between the studies. It is possible that the children in the present sample engaged in more prolonged sedentary time than the previous studies, or that differences found could be due to the different classifications of a prolonged sedentary bout. Future studies should therefore consider developing a universal definition of a prolonged sedentary bout in children. The present study highlights that children aged 11-12-years-old spend 4 hours 25 minutes engaging in prolonged sedentary bouts ≥ 30min, which is

approximately half of their total sedentary time. Strategies may thus be needed to reduce prolonged sedentary time in this age group.

5.2.3 Breaks in sedentary time

The mean number of breaks in sedentary time was 81 per day in the present study. This is similar to results found in children aged 6 – 19 years old who demonstrated 83 breaks per day (Colley *et al.*, 2013). Bailey *et al.* (2016) found children aged 10 – 14 years old engaged in an average of 63 breaks per day, which is less than that found in the present study. A reason for the difference could be the accelerometer used by Bailey *et al.* (2016) could not differentiate posture, thus standing time may have been misclassified as sedentary time (Chastin and Granat, 2009). In addition, Bailey *et al.* (2016) used a 1-min epoch length, which is longer than the 15s epoch used in the present study. Due to children's sporadic and intermittent behaviour (Carson *et al.*, 2014) the longer epoch may not capture all breaks between shorter periods of sedentary time. Future studies should therefore consider using short epoch lengths and the combination of postural allocation and accelerometry to measure children's sedentary behaviour patterns. Girls aged 15 – 18 years old accumulated an average of 55 breaks on a weekday when sedentary time was measured using an activPAL inclinometer (Harrington *et al.*, 2011), which is less than that found in the present study and in the studies by Colley *et al.* (2013) and Bailey *et al.* (2016). A potential reason for the Harrington *et al.* (2011) study demonstrating fewer breaks may be because the participants were older than the children in the present study and the studies by Colley *et al.* (2013) and Bailey *et al.* (2016). In addition, as children get older they tend to take less breaks and spend more time in prolonged sedentary behaviours (Janssen *et al.*, 2016). Janssen *et al.* (2016) reported that the median number of breaks per hour decreased from 8.6 breaks/hour aged 7 years old to 4.1 break/hour aged 15

years old. The present study did not calculate the number of breaks per/hour in sedentary time and thus is difficult to make comparisons between these studies. Future studies should consider interventions that promote the maintenance of the higher number of breaks demonstrated at younger ages, for children as they get older. Despite children in the present study breaking up their sedentary time a total of 81 times per day, approximately half of their total sedentary time was spent in prolonged bouts, meaning the pattern of breaks was not evenly spread throughout the day. Future research should thus examine when the breaks in sedentary time and prolonged sedentary bouts occur to better understand such behaviour patterns in this population

5.3 Sedentary behaviour patterns and cardiometabolic risk

5.3.1 Time in prolonged sedentary bouts and cardiometabolic risk

Time in prolonged sedentary bouts was significantly negatively associated with weight, BMI, WC and body fat% in the regression models in the present study, which was unexpected, as this suggests that children who spend longer periods of time engaging in prolonged sedentary bouts exhibited reduced adiposity levels. Conversely, previous studies have reported positive relationships between prolonged sedentary time and adiposity measures. Altenburg *et al.* (2015) found that prolonged bouts of sedentary time ≥ 30 minutes were significantly positively associated with BMI in children aged 10 – 13 years old, which is in contrast to the present study. Participants in the Altenburg *et al.* (2015) study had a similar mean BMI to the participants in the present study; however, they also had a lower WC. This difference in abdominal obesity may provide reasoning for the difference in findings, although further research is required to establish why. Prolonged bouts of sedentary time ≥ 40 minutes were significantly positively associated with WC and bouts ≥ 80 minutes significantly positively

associated with BMI and WC in boys aged 11 – 14 years old but not in girls of the same age (Colley *et al.*, 2013). This differs from the present study, which may be attributed to the lower weight, but higher BMI and WC characteristics of the participants compared to Colley *et al.* (2013). It is also important to note the definitions of a prolonged sedentary bout are different, thus direct comparisons cannot be made. The results from Colley *et al.* (2013) also suggest that there may be sex differences in the relationship between body composition measures and prolonged sedentary time. Null-associations have also been reported between prolonged bouts of sedentary time ≥ 30 minutes and BMI z-score in children age 10 – 14 years old (Carson *et al.*, 2014). The findings of the present study, and previous studies with opposing findings, suggests that associations between time spent in prolonged sedentary bouts and adiposity remains unclear and suggests and longitudinal studies should thus be conducted to establish causal relationships .

HDL was positively significantly associated with both the time in and number of prolonged sedentary bouts in both regression models in the present study, which was unexpected. This unexpected finding may be influenced by dietary intake (Rocha *et al.*, 2017; Rauber *et al.*, 2015), which was not accounted for in the present study, therefore it is possible that those who engaged in more prolonged sedentary bouts had a diet that encourages higher HDL. Bailey *et al.* (2016) found no significant association between HDL and duration of prolonged sedentary bouts, however, as the present study measured total time in prolonged sedentary bouts rather than mean duration in prolonged sedentary bouts (Bailey *et al.*, 2016) it is difficult to make a direct comparison. In the present study, TC was significantly positively associated with engaging in prolonged sedentary bouts, which may be due to the higher levels of HDL in participants who engaged in more prolonged sedentary bouts. Altenburg *et al.* (2015) found

no significant association between prolonged sedentary bouts of ≥ 20 minutes or ≥ 30 minutes and TC in children aged 10 – 13 years old. A potential reason for the differences could be that in the present study, participants had higher levels of LDL and HDL compared to Altenburg *et al.* (2015), thus increasing TC levels.

In the present study, LDL and non-HDL were significantly positively associated with total time spent in prolonged sedentary bouts, however this association was mediated by MVPA. This indicates that MVPA may protect against high levels of LDL in relation to increased time spent in prolonged sedentary bouts. Previous studies have found that MVPA mediated the associations between sedentary behaviours and cardiometabolic risk markers in children (Chaput *et al.*, 2013; Chastin *et al.*, 2015; Ekelund *et al.*, 2012), thus it is important to consider MVPA when investigating sedentary behaviour and cardiometabolic risk. The present study found no significant associations between the number of prolonged sedentary bouts and triglycerides, TC: HDL ratio, TRG: HDL ratio, glucose, SBP, DBP, HR, clustered cardiometabolic risk score or non-obesity clustered cardiometabolic risk score. No significant associations were found between the time spent in prolonged sedentary bouts and triglycerides, TC: HDL ratio, TRG: HDL ratio, glucose, SBP, DBP, HR, clustered cardiometabolic risk score or non-obesity clustered cardiometabolic risk score. Similarly, Carson and Janssen (2011) found that the total volume of sedentary time and the time spent in bouts of sedentary behaviour ≥ 30 minutes were not independent predictors of SBP or non-HDL. Conversely, other studies have reported that sedentary bouts ≥ 30 minutes were significantly negatively associated with triglycerides in children aged 10 – 13 years old (Altenburg *et al.*, 2015). Comparatively, participants in the present study had higher levels of triglycerides, which may have affected associations with prolonged sedentary bouts. The number of prolonged sedentary bouts has also previously

been significantly associated with hypertriglyceridemia, increased clustered cardiometabolic risk score and increased non-obesity clustered cardiometabolic risk score in children aged 10 – 14 years old (Bailey *et al.*, 2016). However, the duration of prolonged sedentary bouts were not significantly associated with hypertriglyceridemia, increased clustered cardiometabolic risk score and increased non-obesity clustered cardiometabolic risk score (Bailey *et al.*, 2016), although not a direct comparison with the present study. Participants in the study by Bailey *et al.* (2016) had lower body fat%, lower TC, lower HDL, lower triglycerides and a lower BMI z score than those in the present study, which may have impacted the associations with prolonged sedentary bouts and level of clustered cardiometabolic risk. The variance and inconsistency in findings suggests a complex interaction between cardiometabolic risk markers and prolonged sedentary behaviour in children that may be mediated by factors not accounted for in this study, such as diet and the environment. The association between prolonged sedentary time and cardiometabolic markers that adjusts for such variables thus requires further investigation.

5.3.2. Breaks in sedentary time and cardiometabolic risk

In this study, the number of breaks in sedentary time was significantly negatively associated with weight, BMI, WC and body fat%, suggesting that breaking up prolonged sedentary time is beneficial for attenuating cardiometabolic risk markers in children aged 11 – 12 years old. A longitudinal study investigating sedentary behaviour patterns in children, supports the findings of this study; measurements were taken at age 7, 9, 12 and 15 years old, reported an increase in daily breaks in sedentary time between the ages of 9 – 12 years old was significantly associated with a decrease in fat mass index and BMI (Mann *et al.*, 2017). This suggests that

the 9 – 12-year age group could benefit most from breaking up sedentary time to reduce cardiometabolic risk.

In the present study, TC was significantly positively associated with the number of breaks in sedentary time. However, this may be driven by the significant positive association found with HDL, which may thus increase TC as there was no change in LDL. The number of breaks in sedentary time was significantly positively associated with HR, however, this was mediated by MVPA as the association became non-significant when adjusting for this variable. A previous study found that in children aged 8 – 11 years old, breaks in sedentary time was significantly associated with reduced clustered cardiometabolic risk score and lower BMI Z-scores (Saunders *et al.*, 2013b). This is similar, but not a directly comparable, to the present study as breaks in sedentary time were associated with lower BMI scores; however, no significant associations were found between breaks in sedentary time and clustered cardiometabolic risk score. A potential explanation for the differences could be that the children in Saunders *et al.* (2013b) had a higher mean BMI score and higher mean cardiometabolic risk score than in the present study, which may strengthen associations. No significant associations between breaks in sedentary time and triglycerides, LDL, non-HDL, TC: HDL ratio, TRG: HDL ratio, glucose, SBP, DBP were found in the present study. Similarly, Carson and Janssen (2011) found that breaks in sedentary time did not predict high cardiometabolic risk markers in children aged 6 – 19 years old. Furthermore, no significant associations were observed between breaks in sedentary time and blood pressure or non-HDL cholesterol in children aged 6 – 19 years old (Colley *et al.*, 2013). This suggests that breaking up sedentary time, although beneficial for anthropometric cardiometabolic risk makers, may not be enough to affect lipid profile, glucose concentrations and/or blood pressure risk markers in healthy children. Bailey *et al.* (2016) reported no

significant associations between the number of breaks in sedentary time per day and cardiometabolic risk markers; however, the mean duration of daily breaks in sedentary time was beneficially associated with abdominal adiposity and elevated DBP. This suggests that overall, cardiometabolic risk markers in healthy children are moderately stable, thus longitudinal interventions breaking up sitting time may be required to elicit changes in cardiometabolic risk markers in children.

Grace *et al.* (2017) suggest that studies investigating the timing and duration of "breaks in sitting" that lead to improvements in cardiometabolic outcomes are required to inform more specific public health recommendations, particularly in children. Using small amounts of leg movement whilst sitting, such as fidgeting, encourages intermittent increases in vascular shear stress reducing atherogenesis (Morishima *et al.*, 2016). It therefore could be hypothesised that children who fidget whilst sitting down, which is ignored by the activPAL device unless there is movement of the leg (Aminian and Hinckson, 2012), may demonstrate similar benefits as those found in breaking up sitting time. This may provide an explanation as to why fewer significant associations were found between SBP, DBP and HR and TC, HDL, LDL, non-HDL, TC: HDL, TRG: HDL, triglycerides and glucose risk markers and breaking up sitting time in the present study.

5.3.3. Standing and cardiometabolic risk

Standing time was significantly negatively associated with weight and body fat% and significantly positively associated with HDL cholesterol, independent of MVPA. This suggests that increasing standing time may attenuate cardiometabolic risk markers in children aged 11 – 12 years old. A physiological mechanism that may explain this finding is that an increase in standing may elicit a greater expense of daily energy thus decreasing the excess energy that

would be stored as fat (Bailey *et al.*, 2016), thus reducing adiposity levels. WC became significantly negatively associated with standing time when additionally adjusting for MVPA. This suggests that MVPA may mediate the associations between individual cardiometabolic risk markers and standing time in children. No significant associations were found in the in the present study between standing time and BMI, TC, triglycerides, LDL, Non-HDL, TC: HDL, TRG: HDL, glucose, SBP, DBP, HR, clustered cardiometabolic risk score or non-obesity clustered cardiometabolic risk score. The present study highlights that standing may be beneficially associated with adiposity levels in children and should be evaluated as a potential intervention strategy to reduce overweight and obesity.

The present study is the first observational study, to the author's knowledge, to report on the independent associations of standing time with cardiometabolic risk markers in children. Previous studies were unable to report standing time as sedentary time was measured using an accelerometer that could not differentiate posture (Bailey *et al.*, 2016; Colley *et al.*, 2013; Saunders *et al.*, 2013a). The present study provides a rationale to investigate the causal effects of increased standing time on cardiometabolic risk in children to inform the design of interventions to reduce cardiometabolic disease risk in children.

5.4 Strengths and limitations

A strength of this study is the use of an objective measure of sedentary time using the activPAL inclinometer that accurately classifies sedentary time separate from standing. In addition, a wide variety of cardiometabolic risk markers were measured to provide an in-depth exploration of the association between sedentary behaviour patterns and cardiometabolic risk in children. The covariate adjustment used in the present study helped to control for

potentially spurious relationships between variables. Lastly, the narrow age range used in the present study better represents the association of sedentary behaviour patterns with cardiometabolic risk markers for children of this specific age compared with previous studies that have presented findings for samples with larger age ranges (Carson and Janssen, 2011; Bailey *et al.*, 2016; Altenburg *et al.*, 2015; Colley *et al.*, 2013; Martinez-Gomez *et al.*, 2010a; Saunders *et al.*, 2013b). Using larger age ranges may not reflect the sedentary behaviour patterns of children of specific ages within that sample. Future research should therefore consider analysing such associations using narrower age ranges so that recommendations for specific age children can be provided.

One limitation of this study is the cross-sectional design, which limits conclusions of causality (Altenburg *et al.*, 2015) and/or the temporal order of the relationships between sedentary behaviour patterns and cardiometabolic risk markers. Covariates were adjusted for in the present study due to them being related with risk markers, the findings however may have limited generalisability as such relationships may not be consistent across other population samples. It is also important to note that the children in this study accumulated a mean of 90 minutes of MVPA per day, which is 50% higher than the recommended daily amount (World Health Organisation, 2010). In the UK only 32% boys and 24% girls achieve this recommendation (Health and Social Care Information Centre, 2009), making it difficult to generalise the findings of the present study to inactive children. Over a 7-month period, MVPA rather than total sedentary time was shown to predict changes in cardiometabolic risk makers in healthy 10-year-old children (Skrede *et al.*, 2017), thus MVPA should be considered and appropriately adjusted for when investigating sedentary behaviour patterns and cardiometabolic risk. The participants in this sample were from mixed gender state schools in

South East England and thus the findings from this study may not be generalisable to other populations, for instance single sex schools, independent schools and/or other areas of the UK.

A large proportion of the participants in this sample were from least deprived areas based on IMD scores, which although was controlled for in the statistical analysis, mean the findings may not be generalizable to more deprived population groups. In addition, social factors (e.g. cultural and religious influence), environmental factors (e.g. weather conditions, space and equipment/facilities available for active transport or physical activity), and emotional factors (e.g. mood, self-esteem and depressive symptoms) were also unaccounted for in the present study. These factors may affect sedentary behaviour patterns and cardiometabolic risk (Hidding *et al.*, 2017) and thus may influence the associations between sedentary behaviour and cardiometabolic risk markers in the current study.

Diet was not measured in this study to minimise participant burden and maximise compliance, therefore no dietary factors were adjusted for in this study, which could affect the association of sedentary behaviour and cardiometabolic risk markers. The consumption of unhealthy foods (i.e. foods that were ultra-processed, poor in fibre and rich in sodium, fat and refined carbohydrates) has been associated with adverse cardiometabolic risk markers in children and adolescents (Rocha *et al.*, 2017). Consumption of foods rich in fats and refined sugars have been directly associated with increases in lipogenesis, secretion of VLDL and reduced oxidation and greater accumulation of free fatty acids in the blood and tissues (Rauber *et al.*, 2015), which can also lead to increases in weight, WC, BMI and body fat%. Future studies in this area should thus consider controlling for dietary factors.

It has previously been reported that 5% of children will have a blood pressure reading ≥95th percentile from a single visit, however this may reduce to 1% after a second visit due to the child becoming familiarised with the measurement procedure (Gardner and Heady, 1973). Although in the present study the average of two measures was taken, as this occurred during the same visit, it is possible that the children were not familiar with the procedure and thus had increased blood pressure readings due to lack of familiarisation. Furthermore, although the Cholestech LDX® analyzer (Alere San Diego Inc., California, USA) has been used previously in paediatric research (Ahrens *et al.*, 2014; Bailey *et al.*, 2016) and is validated for use in adults (Parikh *et al.*, 2009), to date it has not been validated in a paediatric population. Children typically have low triglyceride concentrations and the Cholestech LDX® analyzer is less sensitive to this measure, often reporting triglyceride levels as < 0.51 mmol · L^{-1}. Therefore, the validity of the glucose and lipid measures may be limited in the present study.

The present study did not measure the context or modality of sedentary behaviours (Tremblay *et al.*, 2017), thus, conclusions regarding the association of specific sedentary behaviours with cardiometabolic risk is unknown. Lastly, there appears to be limited research evaluating the validity and reliability of activPAL weartime in children, thus there is a possibility that the use of a minimum of four days wear and a minimum of 10 h per day weartime may have been insufficient. In addition, the MET cut points for determining PA intensities using the activPAL are manufacturer programmes and validation studies to confirm the appropriateness of these thresholds in children is required.

5.5 Suggestions for further research

It is suggested that future research in this area should employ inclinometer accelerometery to capture true patterns of sedentary time in children. Researchers are encouraged to consider using either overweight/obese participants to investigate the associations of sedentary behaviour patterns with cardiometabolic risk, as these populations tend to spend more time engaged in sedentary behaviours and are more likely to demonstrate cardiometabolic risk markers at an early age (Carson *et al.*, 2016b). In addition, children from different socioeconomic backgrounds should be investigated to elucidate the associations between cardiometabolic risk and sedentary behaviour patterns. Future research should also adjust for dietary factors to account for the relationship that diet may have with cardiometabolic risk and sedentary behaviour in children. Researchers are also advised to familiarise child participants with blood pressure measures before data collection to maximise validity of the data. In addition, the use of validated blood sampling methods in children is recommended to add confidence in the validity of the associations between sedentary behaviour patterns and cardiometabolic risk markers in addition to using more sensitive equipment that would permit detection of a wider triglyceride concentration range.

Future research should consider validating weartime criteria and PA intensities for the activPAL in children of different ages to ensure valid data sedentary time and PA data being recorded for specific age group. Comparisons of different contexts and modalities of sedentary behaviour patterns – i.e. in-school vs. out-of-school time, screen time vs. non-screen time, sitting (active/passive) vs. reclining (active/passive) vs. lying (active/passive) etc. – are under investigated in relation to cardiometabolic risk in children. In addition, it is unknown whether seasonal changes influence patterns of sedentary behaviour.

The present study found that standing was significantly associated with reduced weight, WC and body fat% and increased HDL, thus studies investigating the causal effect of standing on cardiometabolic risk should be conducted. The associations of total sedentary time, prolonged bouts of sedentary time and breaks in sedentary time and cardiometabolic risk are mostly limited to body composition markers in the current study. This may be due to it being more difficult to detect differences in cardiometabolic risk markers in children than in adults is because younger people are more distal to pathophysiological developments (Carson and Janssen, 2011). This means that although children may not exhibit adverse levels of cardiometabolic risk markers there is potential that over time they may accumulate and present later in adulthood. A future study on a population of overweight or obese children may lead to different findings compared to the present study as overweight/obese children would be more likely to exhibit abnormalities in cardiometabolic risk markers than the children in this study (Carson and Janssen, 2011). Longitudinal research and follow ups are limited in relation to investigating the associations of cardiometabolic risk and sedentary behaviour in children, both in observational and intervention studies, thus future research should address this gap in the literature.

6. Conclusion

This study provides evidence that increasing the number of breaks in sedentary behaviour is associated with reductions in weight, BMI, WC and body fat% and increases in HDL in children aged 11 – 12 years old. Increasing standing time is associated with reductions in weight, WC, body fat% and increases in HDL. Total time spent in prolonged sedentary bouts was inversely associated with weight, BMI, WC and body fat% and significantly positively associated with TC and HDL; MVPA mediated associations with LDL and non-HDL.

This study adds to current literature by suggesting that associations between cardiometabolic risk and time spent in prolonged sedentary bouts in children are unclear and requires further research to inform appropriate public health guidelines. Future research should consider investigating different contexts and modalities of sedentary behaviour in relation to associations with cardiometabolic risk in children. The present study provides a rationale for investigating the causal effects of increased standing time on cardiometabolic risk in children to inform the design of interventions to reduce cardiometabolic disease risk in children.

7. References

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8. Appendices

8.1: Parent Information Letter and Consent Form

Institute of Sport and Physical Activity Research
Information Sheet

University of Bedfordshire

The Sit Less for Health study: examining associations of sitting behaviour patterns with heart disease risk in children

Dear Parent/Guardian,

Your child's school has agreed to take part in a school-based research study that will evaluate the relationship between the amount and patterns of sitting and risk markers for heart disease in children. This study is taking place in a number of schools in the Bedford area.

Please read this information sheet carefully before deciding whether your child can take part. If you decide to volunteer we thank you for your participation in this exciting study. If you decide not to take part there will be no disadvantage to you or your child.

What type of participant is needed?
Participants needed for the study are children aged 11-12 years old. Participants will need to meet certain criteria in order to be eligible for the study. In order to ascertain whether your child is eligible, you will need to complete and return the pre-study medical questionnaire and blood analysis form attached, as well as a signed consent form. Participants will be excluded from the study if they have any known blood borne disease, clinically diagnosed diabetes, are taking glucose-lowering and/or lipid-lowering medication, are a smoker, have medically diagnosed hypertension, major illness/injury, or other health issues that may affect them taking part in this study. These documents will be assessed by the research team to ensure participants are suitable to be involved in the study.

What will participants have to do?
Should you agree for your child to take part, your child will be asked to attend a measurement session on one morning before school for us to measure their height, waist circumference using a tape measure, blood pressure and heart rate by inflating a cuff around the arm and then letting the air out slowly using an automatic monitor, and their weight and body fat levels by standing on a scale with two metal plates. We will also take a finger prick blood sample to analyse blood sugar and cholesterol levels. This is a very simple and easy procedure and requires the drops of blood collected to be put into a machine. Your child will be required to fast from 9 pm the night before the measurements are taken and to bring a snack to eat for breakfast after the measurements have been taken. We will also ask you child to complete a short and simple questionnaire about their breakfast habits.

To measure your child's sitting and physical activity behaviours, we will provide them with a small activity monitoring device that will be stuck to the right thigh to be worn for 7 consecutive days. It is attached to the thigh using medical dressing and this will keep the device waterproof. Therefore it can be worn continuously even when bathing or while swimming. It is really important that your child wears this activity monitor continuously every hour of every day throughout the 7 day monitoring period. Without this data we will not know if sitting behaviours are related to heart disease risk markers in children.

We will ask you to provide a contact mobile phone number (either yours or your child's) that a member of the research team can send a text message to during each of the 7 days we are monitoring your child's activity levels to remind them to keep the activity monitor on. This number will remain strictly confidential to the research team only.

What are the possible risks of taking part in the study?
Finger prick blood samples will be collected, which may cause a little discomfort. The blood samples will be taken by a trained researcher following safe practice guidelines and a qualified first aider will be available onsite at all times.

The medical dressing used to attach the ActivPAL device to the skin is very unlikely to cause an allergic reaction, but if the skin is irritated please remove the dressing and inform the research team.

The blood pressure monitor requires the inflation of a cuff around the upper arm which may cause slight discomfort as it tightens but this is very short lasting. All measures will be taken in a private room with only 2-3 children taking part in the study present at any time to reduce any potential feelings of embarrassment. If the child wishes to, they can have their measures taken in privacy from any other children.

What if your child wants to withdraw from the study?
If, at any stage your child wants to leave the study, then they can without needing to give a reason. There will be no disadvantage to your child if they wish to withdraw.

What will happen to the data and information collected?
All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to research team members. Results of the project may be published, but any data included will in no way be linked to any specific participant. Your child will remain completed anonymous. If our results show that your child may have an abnormal heart disease risk marker level, then we will inform you of this and suggest that you book an appointment with your child's GP to discuss this with them.

What if I have any questions?
Please contact the research team (details below) if you have any questions.

Many Thanks,

Miss Stephanie White and Dr Lindsey Smith

Miss Stephanie White

Email: stephanie.white@study.beds.ac.uk

Dr Lindsey Smith
Department of Sport Science and Physical Activity,
University of Bedfordshire
Bedford Campus,
Polhill Avenue,
Bedford

Email: lindsey.smith@beds.ac.uk

Tel: 01234 793093

Institute for Sport and Physical Activity Research
Parent / Guardian Consent form

University of Bedfordshire

The Sit Less for Health study

Please circle as appropriate:

Have you received, read and understood a copy of the Information Letter?	Yes	No
Do you understand that your child's participation in this study is entirely voluntary?	Yes	No
Do you understand that you are free to refuse your child's participation and have the right to withdraw at any time for any reason without it influencing your child, and that all data collected from you at that time will be removed?	Yes	No
Do you understand that your child's name WILL NOT be shared or disclosed in the reporting of results?	Yes	No
Do you understand that your child's name will not be displayed in any reports, presentations or publications?	Yes	No
Do you confirm that you have had an opportunity to ask questions and that your questions have been answered to your satisfaction?	Yes	No
Do you understand that measurements will be taken by researchers who will each have DBS clearance?	Yes	No
Do you understand that the measurements taken will include your child's height, weight, waist circumference, body fat levels, heart rate, blood pressure, blood sugar levels, cholesterol levels, physical activity and sitting behaviour, and completion of an activity log book and breakfast habits questionnaire?	Yes	No
Do you understand that a finger prick blood sample will be taken in order to measure your child's blood sugar and cholesterol levels?	Yes	No
Do you understand that the ActivPAL device must be worn for one week and must only be removed if instructed to by a researcher or if an allergic reaction occurs?	Yes	No

Do you understand that your child will receive a daily text message to the phone number you provide to remind them to wear their ActivPAL device each day for one week?. This mobile phone number provided will only be used for this purpose and will not be shared with anyone outside of the research team. Yes No

Do you understand that on completion of the study and the safe return of their activity monitor your child will receive a £5 shopping gift voucher? Yes No

Do you give full informed consent for your child to take part in this research project? Yes No

Name of child: _____

Name of Parent/Guardian: _____

Contact Telephone Number: _____

Parent/Guardian Email Address: _____

Mobile phone number for text reminders: _____

Whose number is this? (please circle): Parent/Guardian / Child

Home Address (of child): _____

_____ Post code: _____

Parent/Guardian Signature: _____

Date: _____

Along with this form, please also return the following completed forms:

Pre-test medical questionnaire and blood analysis participant screening form

Thank you for your participation! **Please complete and return these forms to [Insert Person] by [Insert date]**

University of Bedfordshire

Institute for Sport and Physical Activity Research
Polhill Avenue
Bedford
MK41 9EA

PRE-TEST MEDICAL QUESTIONNAIRE

To be completed before any measurements are taken.

Child's Name: _____

Date of Birth: _____ Gender: Male / Female

1 Is your child in good health? Yes / No

If no, please explain:

2 Has your child suffered from a serious illness or accident? Yes / No

If yes, please give particulars:

3 Is your child recovering from an illness or operation? Yes / No

If yes, please give particulars:

4 Does your child suffer, or ever suffered from:

Respiratory conditions (asthma, bronchitis, tuberculosis, other)? Yes / No

Diabetes? Yes / No

Epilepsy? Yes / No

High blood pressure? Yes / No

Heart conditions or circulation problems: (please circle)

(angina, high blood pressure, varicose vein, aneurysm, embolism, heart attack, other)?

Chest pains at any time? Yes / No

Fainting/blackouts/dizziness? Yes / No

Is there any history of heart disease in your family? Yes / No

5 Is your child currently taking medication? Yes / No

If yes, please give particulars:

6 Is your child currently attending your GP for any condition or has your child consulted your doctor in the last three months? Yes / No

If yes, please give particulars:

7 Has your child had to consult your doctor, or had hospital treatment within the last six months? Yes / No

If yes, please give particulars:

Name of child (please print) _____

Name of Parent/Guardian (please print) _____

Signature of Parent/Guardian _____ Date: _____

Child's Name: _____

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?
b. Have you had jaundice within the previous year?
c. Have you ever had any form of hepatitis?
d. Have you any reason to think you are HIV positive?
e. Have you ever been involved in intravenous drug use?
f. Are you a haemophiliac?
g. Is there any other reason you are aware of why taking blood might be hazardous to your health?
h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer Yes to any of questions a-g? Please tick your response.

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Signed .....

Date .....

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

The completed Medical Questionnaire (Par Q) and this Blood Sampling Form will be held in a locked filing cabinet in the School of PE and Sport Sciences laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

If you wish to have a photocopy of any of the completed documents, please ask for one.

8.2: Email to schools

Dear _____,

We have some exciting new research starting at the University of Bedfordshire and we would like to partner with local schools to conduct this very important research.

The research project will investigate the associations of prolonged sitting and health risk markers in children aged 11-12 (Year 7). More children than ever are being diagnosed with diseases typically associated with adults, such as Type 2 diabetes and high cholesterol levels, and one potential reason for this could be due to the sedentary nature of life in the 21st Century.

We would like to offer your students the opportunity to discover how much time they spend being inactive and/or sat down in a week and to provide them with a simple health check. We would require participants to wear a small device on their leg for one week that monitors their movements, which is discrete and waterproof. In addition, we would like to come into school and take some measurements from them such as height, weight, body fat level, blood sugar and cholesterol levels (obtained through a small finger prick blood sample) and for students to complete some short questionnaires. We intend for the measurements to be taken before school so that their learning is not compromised.

We will be able to produce a summary report for the school on the activity levels and health status of students which may be of interest to OFSTED and staff involved in health and wellbeing policies. In addition, we are willing to provide a 1-hour CPD workshop for your staff

on the results from the study and potential strategies that could be used in the classroom to enhance student's health, without compromising learning.

If you would like to be involved in this new study or would like to find out more, please contact me or Dr Lindsey Smith (lindsey.smith@beds.ac.uk) for information.

We look forward to hearing from you.

Kind regards,

Stephanie

8.3: School Assembly PowerPoint

8.4: Reminder Letter

Institute for Sport and Physical Activity Research
Reminder Letter

University of Bedfordshire

The Sit Less for Health study: examining associations of sitting behaviour patterns with heart disease risk in children

Dear **[CHILD NAME]** and Parent/Guardian,

Thank you for agreeing to take part our exciting school-based study. This letter is to let you know that we will be coming to your child's school to take measures from them on **[Insert Date] at [Insert Time] in room XXX** . Please remember to do the following before coming to have your measures taken:

- Please wear your PE kit (including shorts)
- Fast from 9 pm the evening before (do not eat anything). You can sip small amounts of water.
- Do NOT eat breakfast or drink anything other than small sips of water on the morning of your measurements
- Bring a snack to eat for breakfast AFTER the measurements have been taken

At the end of the measurement session, you will have an activity monitor stuck to your right leg which you must wear continuously for 8 days. We will come back to your school to remove it on **[Insert Date]** (we will give you further instructions about this). Your will also need to complete an activity diary each day that we will provide you with. Please return this when you return the activity monitor. Remember that we will also send you a text each morning to remind you to keep the activity monitor on!

On completion of the study and the safe return of the activity monitor and activity diary, you will receive a £5 shopping voucher as a thank you for taking part in the study.

If you have any concerns or questions, please contact Stephanie White or Dr Lindsey Smith (contact details below).

Many Thanks,

Miss Stephanie White and Dr Lindsey Smith

Miss Stephanie White
Email: stephanie.white@study.beds.ac.uk Tel: 07792650485

Dr Lindsey Smith
Email: lindsey.smith@beds.ac.uk Tel: 01234 793093

8.5: Reminder Text Messages

8.5.1: Day before measurement morning reminder

Dear Volunteer. This is a reminder that the Sit Less for Health Study measurement morning is tomorrow [Insert Date]. Please arrive at the time stated on your letter. Don't forget that you cannot eat or drink (except small amounts of water) from 9pm tonight and do not eat breakfast in the morning – bring it with you to school! Please do not reply to this message. If you have any questions please email stephanie.white@study.beds.ac.uk

8.5.2: Daily reminder

Daily Reminder Dear volunteer. Please wear your activity monitor at all times! Only take it off if you are swimming or going in the sea. You CAN wear it to shower or bathe and when playing sports. Make sure you complete your activity diary today! Thanks, the Sit Less for Health Study Team.

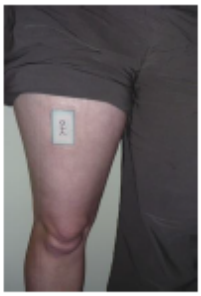
8.5.3: Final daily reminder

Last daily reminder Tomorrow is the day we will collect your activity monitor and diary so please make sure you bring them with you to school! Please come to [insert venue] at the beginning of lunch time [insert time] to hand these back and to collect your amazon voucher. Thanks, the Sit Less for Health Study Team.

8.6: Activity log

Thigh Monitor Instructions

How do I wear the monitor?



- The Thigh Monitor is attached directly onto the skin and positioned on the front of the thigh, roughly half way between the hip and knee with the stick man standing up (see picture).
- Please wear the monitor **every day for 8 days**
- Please wear the Thigh Monitor continuously (24 hours/day)
- The Thigh Monitor can be worn during sleep and is water resistant so you can wear it whilst showering and bathing but please do not wear it in the swimming pool in case it falls off.
- The adhesive patch that sticks the Thigh Monitor to your skin may last up to 8 days but if it comes loose you should replace it.

Note: The Thigh Monitor will emit a green flash every 6 seconds. This is an indication that it is working and recording data.

How do I change the adhesive patch?

- Remove the Thigh Monitor from your thigh (note that this may cause some slight discomfort) and peel the adhesive patch off the Thigh Monitor. The monitor is covered in a waterproof sleeve and wrapped in one adhesive patch—please make sure that these remain on the monitor when you do this.
- With an alcohol wipe provided in your Activity Monitor Pack, thoroughly wipe down the monitor and the area of your leg where the Thigh Monitor was attached.
- Position the Thigh Monitor in the same spot as before on your thigh (or on the other thigh if you have had a slight irritation), ensuring that the stick man on the front of the Thigh Monitor is standing up (head facing upwards).
- Peel the backing off an adhesive patch and place it over the Thigh Monitor. Press the patch onto your skin, peel back the top layer of the patch and smooth out the air bubbles and wrinkles as much as possible to ensure that the Thigh Monitor is firmly secured to your thigh.
- If you require assistance re-attaching your Thigh Monitor, or if you experience any skin irritation whilst wearing it, please call Stephanie White on 07792 650485.

What else do I need to do?

- It is important that you fill in the **Daily Log** on the following pages every day for the 8 days while you are wearing the monitor.
- This helps us to know when you were awake and asleep.

Returning your Thigh Monitor and Daily Log

- Please return your thigh monitor to **Stephanie (Lead Researcher) on** _____ along with this completed Daily Log and any unused adhesive patches for the research team to collect.

How to fill in the Daily Thigh Monitor Log

- The log is divided into 8 days. Please complete each question for all of the eight days. Please try and be as accurate as possible—record the exact times if you can, or at least to the nearest 5 minutes of your estimated times.

- Start by writing the **date** in the top row.

- Then record the time that you **woke up** and the time that you actually **got out of bed** (these times may be the same for some days). We ask for these two times because people sometimes spend time in bed before going to sleep or getting up. We are interested in knowing actual sleeping time and time in bed before sleep or after waking up for example going to bed and watching TV for an hour before going to sleep.

- If you **remove the device** for longer than 10 minutes during the day please note down the time that you removed the device, the time that you re-attached it and the reason why you removed the device. This is important as we cannot tell from the data if you are lying down or whether you have removed the device and are just not wearing it (the data looks the same when we look at it).

- Then record what time you **got into bed** to go to sleep and the time that you actually **went to sleep.** So put the rough time that you fell asleep and not the time that you got into bed. This is important as the monitor cannot tell the difference between asleep and awake times, and we are only interested in your activity while you are awake.

- Please record your sleep time first thing in the morning when you wake up along with your wake time.

- There is also a space for you to make **comments**. It is useful for us to know if you have had any skin irritations, accidentally worn the monitor upside down or any other information that you think we should know.

- Once you have completed your 8 days of wear please return this log along with your thigh monitor.

If you have any questions please contact Stephanie White on 07792 650485

Thigh Monitor Log

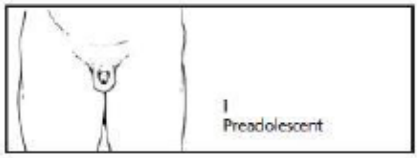
Day and Date	Time woke up	Time got out of bed	Did you remove your monitor for more than 10 mins today?	If removed, record time of removal and reason why	Time got into bed	Time went to sleep	Other comments	Did you have breakfast (more than a glass of milk or fruit juice) today?
Example Day 1 17/12/2013	*07:00am*	*07:15am*	*Yes* (circled) *No*	*Time off: 18:00pm* *Time on:18:45pm* *Reason: Swimming in the sea*	*20:45pm*	*21:10pm*	*Slight irritation on right leg so put monitor on left leg*	*Yes* (circled) *No*
Day 1 Date:			Yes No					Yes No
Day 2 Date:			Yes No					Yes No
Day 3 Date:			Yes No					Yes No
Day 4 Date:			Yes No					Yes No
Day 5 Date:			Yes No					Yes No
Day 6 Date:			Yes No					Yes No
Day 7 Date:			Yes No					Yes No
Day 8 Date:			Yes No					Yes No

8.7: Biological Maturity Questionnaire

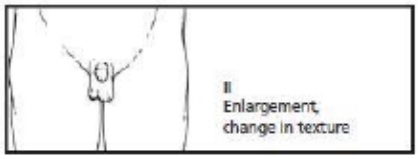
8.7.1: Biological Maturity Pictures (Males)

1. GENITALIA DEVELOPMENT (MATURITY RATINGS)

The pictures on this page show the different stages of development of the male penis. A boy passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form.

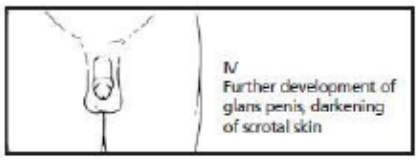


Stage 1
The testes, scrotal sac and penis are similar in size and proportion to those seen in early childhood.

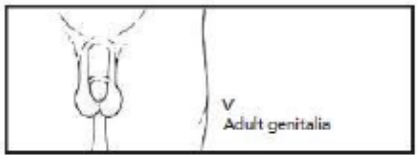


Stage 2
There is enlargement of the scrotum and testes and a change in the texture of the scrotal skin. The scrotal skin may also look slightly red.

Stage 3
Growth of the penis has occurred, initially in length, although with some increase in circumference. There is also increased growth of the testes and scrotum



Stage 4
The penis is significantly enlarged in length and circumference. The testes and scrotum continue to enlarge, and there is darkening of the scrotal skin



Stage 5
The genitalia are adult with regard to size and shape.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of boys involved in the study, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form, they will never be available to anyone not involved in the study.

PLEASE TURN OVER

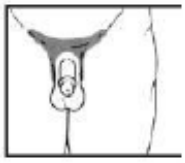
2. PUBIC HAIR DEVELOPMENT (MATURITY RATINGS)

The pictures on this page show different amounts of male pubic hair. A boy passes through each of the five stages shown by these drawings. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form.

Stage 1
There is no pubic hair at all.

Stage 2
There is a little soft, long, hair. This hair may be straight or a little curly and covers a small area.

Stage 3
The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a larger area.



Stage 4
The hair is now as dark, curly, and coarse as that of an adult male. However, the area that the hair covers is not as large as that of an adult male. The hair has not spread out to the thighs.

Stage 5
The hair has spread out to touch the thighs. The hair is now like that of an adult female. The hair usually forms a triangle pattern (▼) as it spreads out to the thighs.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of boys involved in the study, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form, they will never be available to anyone not involved in the study.

Please do not send this sheet back with your response (answer) sheet

8.7.2: Coded maturity response (Males)

Code _____

Boys

Please circle the number where the written description and picture is most like you. You need to provide two responses (refer to the scientific pictures given to you). Please remember that none of the people that you have met at the University will be able to link this information directly to you.

1.	GD	1	2	3	4	5
2.	PH	1	2	3	4	5

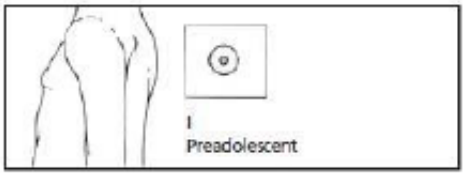
Thank you for answering the questions. Please put the completed sheet in the envelope and give it to one of the researchers when they come to your school. All of your answers and responses are completely confidential and would have no meaning to anyone not involved in the study.

Please do not send the photograph sheet back with your response (answer) sheet

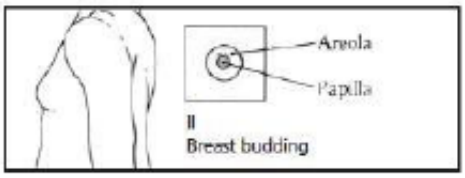
8.7.3: Biological Maturity Pictures (Females)

1. BREAST DEVELOPMENT (MATURITY RATINGS)

The pictures on this page show the different stages of development of the female breast. A girl passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form.

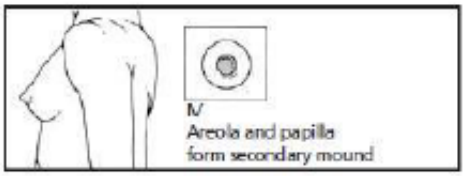


Stage 1
The nipple is raised a little in this stage. The rest of the breast is still flat.

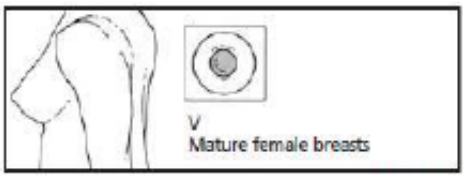


Stage 2
The papilla is raised more than in stage 1. The breast is a small mound. The areola (coloured skin around the nipple) is larger than in stage 1.

Stage 3
The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast.



Stage 4
The areola and papilla rise above the level of the breasts and form secondary mounds with further development of the overall breast tissue. This stage may not happen at all for some girls, some girls will develop from stage 3 to stage 5 with no stage 4.



Stage 5
This is the mature adult stage. The breasts are fully developed, only the nipple sticks out in this stage. The areola has moved back to the general shape of the breast.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of girls involved in the study, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form, they will never be available to anyone not involved in the study.

PLEASE TURN OVER

2. PUBIC HAIR DEVELOPMENT (MATURITY RATINGS)

The pictures on this page show different amounts of female pubic hair. A girl passes through each of the five stages shown by these drawings. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form.

Stage 1
There is no pubic hair at all.

Stage 2
There is a small amount of long soft hair, which may be straight or a little curly. These hairs are mainly along the labia

Stage 3
The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a larger area.

Stage 4
The hair is now as dark, curly, and coarse as that of an adult female. However, the area that the hair covers is not as large as that of an adult female. The hair has not spread out to the thighs.

Stage 5
The hair has spread out to touch the thighs. The hair is now like that of an adult female. The hair usually forms a triangle pattern (▼) as it spreads out to the thighs.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of girls involved in the study, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form, they will never be available to anyone not involved in the study.

Please do not send this sheet back with your response (answer) sheet

8.7.4: Coded maturity response (Females)

Code _____

Girls

Please circle the number where the written description and picture is most like you. You need to provide two responses (refer to the scientific pictures given to you). Please remember that none of the people that you have met at the University will be able to link this information directly to you.

1.	BD	1	2	3	4	5
2.	PH	1	2	3	4	5

Thank you for answering the questions. Please put the completed sheet in the envelope and give it to one of the researchers when they come to your school. All of your answers and responses are completely confidential and would have no meaning to anyone not involved in the study.

Please do not send the photograph sheet back with your response (answer) sheet